Volume 47, No. 3
March 1959

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

TRANSLATED FROM RUSSIAN

CONSULTANTS BUREAU, INC.

RUSSIAN PHARMACEUTICAL RESEARCH
IN COMPLETE ENGLISH TRANSLATION

SOVIET PHARMACEUTICAL RESEARCH

Chemistry Collection No. 4

Covers all aspects of Soviet pharmaceutical research; papers selected by one of the top pharmaceutical research chemists, of one of the major drug companies, from all Russian chemistry journals translated by Consultants Bureau, **1949–1955.** Sections may be purchased as follows:

I. PHARMACEUTICAL CHEMISTRY. Sections: Solubility; Stability: Ion Exchange; Emulsions, Suspensions, Gels; Miscellaneous. 74 papers, 447 pages, **$95.00**

II. PHARMACOGNOSY. Sections: Alkaloids; Oils; Glycosides; Miscellaneous. 87 papers, 399 pages, **$90.00**

III. MEDICINAL CHEMISTRY. Sections: Structure-Activity Relationships; General. 91 papers, 556 pages, **$100.00**

THE COMPLETE COLLECTION, 252 papers, 1,402 pages, **only $200.00**

*Single articles available — Table of Contents sent on request

All Consultants Bureau's translations by bilingual scientists, equally familiar with Russian and the technical terminology. All diagrammatic and tabular material integral with the text is included; text is clearly reproduced by multilith process from IBM cold type; books are staple bound in durable paper covers. Complete catalogs of Consultants Bureau translations available

CONSULTANTS BUREAU, INC.

227 WEST 17TH STREET, NEW YORK 11, N. Y

Volume 47, No. 3
March 1959

BULLETIN OF

EXPERIMENTAL BIOLOGY AND MEDICINE

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

A publication of the Academy of Medical Sciences of the USSR

Year and issue of first translation:
Vol. 41 No. 1 January 1956

Annual Subscription	*$20.00*
Single Issue	*10.00*

TRANSLATED FROM RUSSIAN

Copyright 1959

CONSULTANTS BUREAU, INC.
227 W. 17th St., NEW YORK 11, N.Y.

EDITORIAL BOARD OF
BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

A. Ya. Alymov, N. N. Zhukov-Verezhnikov (Acting Editor), S. Ya. Kaplanskii, G. N. Kryzhanovskii (Secretary), M. N. Livanov, L. D. Liozner, I. N. Maiskii, V. A. Negovskii, V. V. Parin (Acting Editor), V. A. Sanotskii, A. A. Solov'ev.

The National Institutes of Health of the Public Health Service has supported in part the cost of publishing BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE through the purchase of copies for distribution to American medical libraries.

Note: The sale of photostatic copies of any portion of this copyright translation is expressly prohibited by the copyright owners.

Printed in the United States of America

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

Volume 47, Number 3 March 1959

CONTENTS

	PAGE	RUSS. PAGE
PHYSIOLOGY		
Conditioned Reflex Changes in the Human Electroretinogram. A. I. Bogoslovskii, and E. N. Semenovskaya	265	3
The Effect of Sulfhydril Groups on Veratrine Sensitization of Muscle to the Action of Potassium. Kh. S. Koshtoyants and L. S. Bunkina	270	8
Effect of Temperature on the Excitability of Pigeon Muscle. I. P. Suzdal'skaya	272	11
Vasomotor Regulatory Mechanisms. Communication III. Reflexes Involving Efferent Pathways to the Vessels of the Limbs and Afferents from Somatic Nerves. V. M. Khayutin and V. L. Tsaturov	277	16
PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY		
The Role of the Adrenals in the Restoration of the Vital Functions After Clinical Death. M. G. Kolpakov, M. G. Polyak and G. S. Yakobson	282	21
The Effect of Pituitrin on the Development of Experimental Atherosclerosis. A. A. Belous	289	27
Experimental Induction of Atherosclerosis in Rabbits. N. A. Yushchenko	293	31
BIOCHEMISTRY AND BIOPHYSICS		
Changes in the Ammonia and Glutamic Acid Contents of the Brain of Animals Following Surgical Interference with Liver Function. N. V. Veselkin and B. G. Gordon	296	34
Mitogenetic Analysis of the Protein Substrate of Protoplasm. Part II. Dividing Cells and Cells which have Emerged from the "Meristematous" State. N. S. Slavina	301	39
Effect of Ionizing Radiation on the Uric Acid and Allantoin Contents of the Blood and Urine of Animals. T. A. Fedorova, V. P. Fedotov and N. A. Mkrtumova	306	44
Reduction of Methemoglobin by Means of Glutamic Acid. A. M. Genkin and M. S. Volkov	312	50
PHARMACOLOGY		
Changes in the Level of the Arterial Blood Pressure in Dogs Due to Combinations of Hypotensive Drugs. N. P. Speranskaya	315	53
The Importance of the Pituitary-Adrenal System in the Sensitivity of Animals to Narcotics at Various Ages. I. V. Markova	320	57
Changes in the Interoceptive Reflexes in Dogs Under the Influence of Narcotics. T. V. Popova	324	61

CONTENTS (continued)

	PAGE	RUSS. PAGE
MICROBIOLOGY AND IMMUNITY		
Impulses in the Sensory Nerves in Response to Stimulation of the Skin Receptors of Immunized Animals with Antigen. A. N. Gordienko, B. A. Saakov and I. M. Bondarev	329	66
ONCOLOGY		
A Comparative Study of the Serological Properties of Fractions of a Cancer Antiserum from Horses. N. A. Nazarenko and V. S. Gostev	333	70
EXPERIMENTAL BIOLOGY		
Morphological Changes in the Jejunum and Ileum of Rats After Wide Resection of the Small Intestine. N. P. Bochkov	339	76
The Regeneration of Skeletal Muscle in Mammals Under Novocain Block. N. I. Grebenskaya	344	81
The Effect of a Fall of the Temperature on Regeneration in the Amphibia and on the Resistance of the Regenerating Tissue Under these Conditions to the Cytotaxic Action of Novembichin and TEM. E. Ch. Pukhal'skaya	348	85
The Regenerative Power of the Hypertrophied Lung of the Crested Triton. L. K. Romanova	352	89
The Mitotic Activity of the Cornea in Mice After Infliction of a Burn at Different Times of Day. M. T. Gololobova	358	94
A Comparative Study of the Antigenic Properties of the Tissues of Different Species of Animals at Various Stages of Development. Communication III. A Comparative Study of the Antigenic Properties of the Heart Tissue of the Frog (Rana temporaria) and the Triton (Triturus taeniatus). R. F. Averkina	361	97
The Use of Cortisone and of Long-Acting ACTH to Assess the Biological Activity of the Somatotropic Hormone (Growth Hormone). I. A. Eskin and N. V. Mikhailova	367	103
MORPHOLOGY AND PATHOMORPHOLOGY		
Data on the Innervation of the Mucous Membrane of the Stomach in Mammals and Man. F. D. Gilev	372	109
Changes in the Lung after Ligation of the Pulmonary Artery and Introduction of a Foreign Body into the Trachea. N. V. Voikova	376	112
METHODS		
A Method of Determination of the Oxygen Demand of Cardiac Muscle by Means of the Kreps Oximeter. I. E. Kisin	381	117
The Detection of Dead and Living Cells by the Method of Fluorescent Microscopy. P. V. Breivis and Yu. M. Vasil'ev	384	119
A Method of Recording the Ballistocardiogram of Acceleration. I. E. Oranskii and N. F. Smirnov	389	123

PHYSIOLOGY

CONDITIONED REFLEX CHANGES IN THE HUMAN ELECTRORETINOGRAM

A. I. Bogoslovskii and E. N. Semenovskaya

From the S. V. Kravkov Laboratory of Physiological Optics (Head – Candidate of Medical Sciences A. V. Roslavtsev) of the Helmholtz State Scientific Research Institute of Ophthalmology (Director – Candidate of Medical Sciences A. V. Roslavtsev), Moscow

(Received March 31, 1958. Presented by Active Member of the AMN SSSR V. V. Parin)

The possibility of action of the higher nervous centers on processes taking place in the retina, has not yet been adequately established. E. M. Belostopkii, A. I. Bogoslovskii and E. N. Semenovskaya (1957) found in one case of traumatic encephalopathy, in which there was marked reduction in visual acuity and concentric reduction of the field of view to 10°, that the amplitude of the elctroretinogram (ERG) was greater than normal. They found however, that the amplitude of the b wave in subjects with normal vision varies from one experiment to another until it attains a fairly stable value. A. I. Bogoslovskii and E. M. Semenovskaya found in a case of complete blindness of one eye due to the growth of an intracranial tumor, that the amplitude of the b wave in this eye was greater than in the other, in which full vision was preserved and in which the ERG was normal. R. B. Zaretskaya [1] found that caffeine and bromine increase the human ERG. These facts lead us to suppose that although the ERG is most probably an expression of the electrical activity of the retina, and only arises from it, nevertheless it is under the control of the higher visual centers. This would certainly appear to be so from the theoretical point of view, but until recently the fact has not been demonstrated experimentally. Only I. Vaný̌sek [3] has described experiments from which it can be seen that the central nervous system influences the human ERG. He measured the ERG in 11 healthy subjects during the course of dark adaptation. He found that dark adaptation increases the amplitude of the b wave. At the 12th minute of dark adaptation, the subject was presented with a bright light. The light adaptation caused a reduction in the amplitude of the b wave in 6 out of the 11 subjects, and this reduction could be induced by one single presentation of the light. Vaný̌sek quite correctly concludes that the human ERG may be influenced by the central nervous system. Finally, in her article, A. V. Zonova [2] describes conditioned reflex changes in the ERG of the rabbit. The unconditioned stimulus used was subconjunctival injection of sodium chloride, and the conditioned stimuli were a sound and proprioceptive stimulation.

In the present investigation we have employed a different method of demonstrating the regulatory effect of the central nervous system on the ERG. We have tried to bring about conditioned reflex changes in the human ERG in response to an initially indifferent stimulus, while for the conditioned stimulus we have used the adequate stimulus – light.

METHOD

The electroretinogram was recorded using a silver electrode fixed to a contact lens. A silver disk fixed by a plastic clip to the lobe of the homolateral ear was used as the indifferent electrode. Simultaneous recordings were made of the elctroencephalogram (EEG) of both occipital areas. To record ERG and EEG an MPO-2 string oscillograph and the six-channel amplifier system of Peimer and Ul't were used.

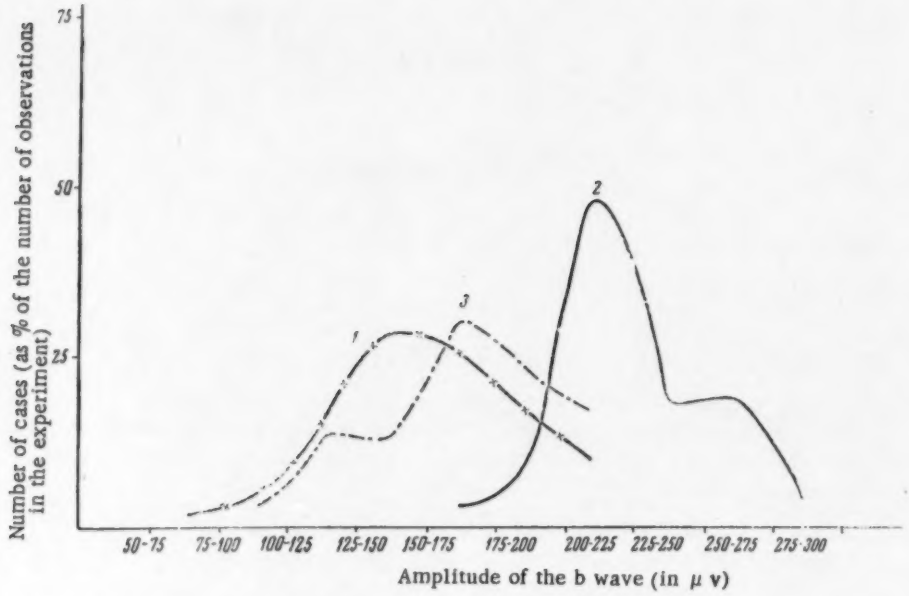


Fig. 1. Distribution curves for the amplitude of the b wave of the ERG.
1) In response to light giving a brightness at the eye of approximately 80 lux (weak light); 2) in response to combined sound + strong light stimulus (giving a brightness at the eye of about 600 lux); 3) in response to combined stimulus of sound + weak light (80 lux).

The light stimulus subtended about 1.5° at the eye. Two different brightnesses of stimulus were used, the weaker giving a brightness of 80 lux at the pupil, and the stronger a brightness of about 600 lux. As an auditory stimulus, we used the sound of a ZG-10 generator having a loudness of 60 decibels above threshold, and a frequency of 250 c/s. The sound was emitted from a loudspeaker placed 1 m in front of the subject. It had no direct effect on the ERG. Two subjects were used for the experiments: a woman M, of 45 years, and a man O, 30 years old. Neither had any ophthalmological defect. The visual acuity in both subjects was 1.0.

In preliminary experiments, we determined the qualitative and quantitative features of the ERG and EEG in response to the weak light of 40 milliseconds duration, and to the strong light, using the same exposure, and to the combined stimulus of sound + strong light. Initially the sound was switched on and maintained for 30 seconds, and during the 15th second of the sound, the strong light of 40 milliseconds duration was presented. The experiment was conducted as follows: The electrodes were placed in position. The subject was placed in total darkness for 3 minutes; while sitting comfortably in an armchair, he directed his attention to a red fixation point placed adjacent to the test field.

Preliminary tests showed that the response of the ERG and EEG to these stimuli was very constant during the single experiments, and showed very little variation from one experiment to another. For instance, for the subject M, on stimulating with the weak light, the ERG produced a b wave with an average amplitude of 135 μv; the latent period of the b wave was about 60 milliseconds; the duration of the b wave was, on average, about 200 milliseconds. On stimulating with the strong light, the amplitude of the b wave was about 225 μ v; the latent period was about 40 milliseconds, and the total duration about 160 milliseconds. When the sound was given together with the stronger light stimulus, the ERG was the same as to the strong light alone. After the preliminary tests had been concluded, we proceeded to the main experiment.

Here the sequence was as follows. After the subject had been in darkness for 3 minutes, and while fixating the red point, the following stimuli were presented in a random order at intervals of 30 seconds: weak light (80 lux; 2-3 presentations at intervals of 15 seconds) or a 15-second sound stimulation on which were superimposed 2-3-4 strong light flashes of 600 lux at intervals of 15 seconds. This procedure is called "elaborating aconditioned reflex" to sound; it was repeated many times during the experiment, which lasted altogether about 2 hours. Each time recordings of both ERG and EEG were made.

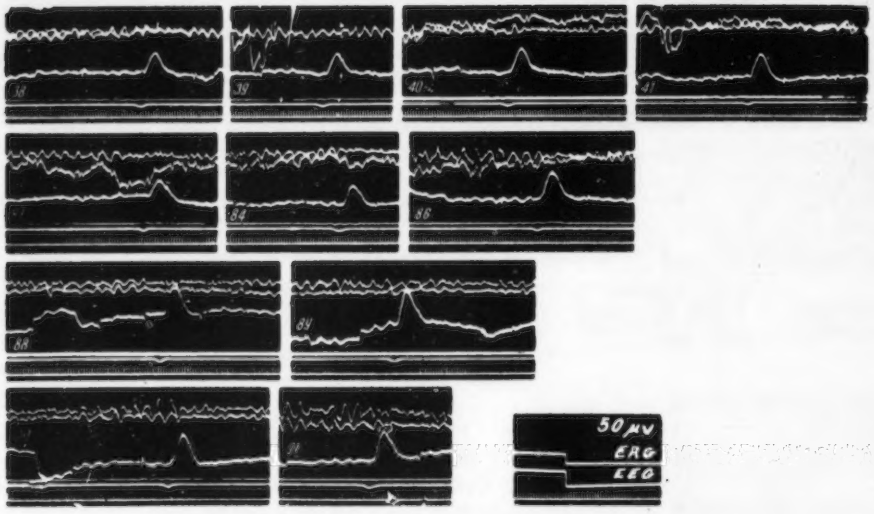


Fig. 2. Changes in the ERG in response to conditioned reflex stimulation. Curves, from above downwards: EEG of right occipital area, EEG of left occipital area, ERG, trace of sound signal (switching on sound causes broadening of line. See traces 40, 41, 88, and 89), record of light stimulus (downward displacement at time of light stimulus). Time marking 40 milliseconds. Calibration 50 μv; upper cal. for ERG, lower, for EEG.

After a few coincidences of the bell + strong light, instead of the strong light being superimposed on the 15-second sound stimulation, the weak light was substituted. This procedure was known as "testing the conditioned reflex". Both before and after this testing of the reflex, the weak light was presented alone as control.

We assumed that if it were possible to elaborate a conditioned reflex change in the ERG, then such a procedure would produce a change in the ERG in response to the combination of sound + weak light which would approximate that of the ERG in response to the combination of sound + strong light. Since a simultaneous record of the EEG was made, it would be expected that the response in it to the sound would also show conditioned reflex changes; however, we did not investigate this point, since such changes have been known for a long time (G. Jasper, I. S. Beritov, A. Gasteau, V. S. Rusinov, and others).

RESULTS

We will give the detailed results only for the subject M, since those obtained with subject O were similar. With subject M, we carried out a total of 15 experiments during July of 1957. 628 separate ERG and EEG recordings were obtained, and of these 242 were recordings of the combined effect of sound + strong light ("development of the conditioned reflex"), 71 showed the response to sound + weak light ("testing the conditioned reflex"), and 315 were recordings of the response to weak light alone (control).

In examining the results, we first paid attention to the amplitude of the b wave of the ERG, then to its latent period, time to reach maximum value, and to its total duration. With the combined stimuli of sound and strong light, the b wave amplitude had an average value of 207 μv, in response to the weak light alone the value was 144 μv, and to the combined stimulus of sound and weak light ("testing the conditioned reflex") it was 158 μv. A still greater difference was shown between the second and third combinations, as can be seen by comparing the results of the first and of the second sets of experiments. In the first set, the amplitude of the b wave in response to the weak light alone was, on average, 135 μv, while to the combination of sound + weak light the average value was 139 μv. However, in the second set, the corresponding values were 140 and 166 μv. Fig. 1 shows curves giving the variation in amplitude of the b wave for the three conditions used. Despite the comparatively small number of readings, the curves show a fairly typical Gaussian distribution. The modal value for curve 1 is 138, for curve 3 – 167, and for curve 2 – 225 μv.

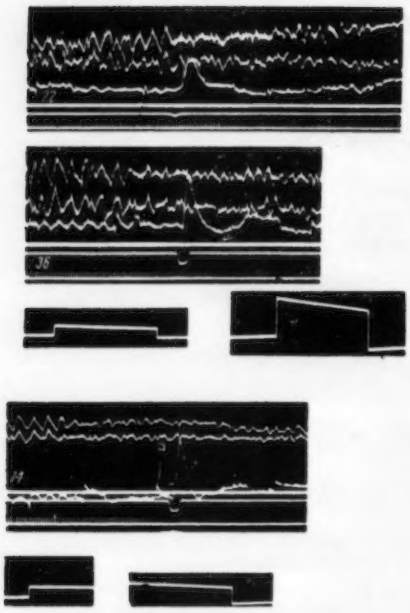


Fig. 3. EEG during elaboration of conditioned reflex changes in the ERG. Sequence of traces as in Fig. 2. In response to the combination of a strong light and a sound stimulus, the α-rhythm is arrested before the light flash is given (see curve 36 and 14). Calibration 50 μv, left cal. for ERG, right, for EEG.

From Fig. 1 it can be seen that the whole of the curve and the mode for the combination of sound + weak light is displaced in the direction of the maximum of the curve for sound + strong light. This indicates that in our experiments, the sound has acquired the significance of a signal, and that it affects the ERG by changing the amplitude of the b wave in accordance with the principles of conditioned reflex action. This change was not great, but it was greater than any experimental error. The conditioned reflex change in the ERG can be seen still more clearly in the curves obtained when the sound had already come to acquire the significance of a signal (Fig. 2). All these curves were obtained from an experiment made on 7/19/1957. Here it emerges very definitely that the amplitudes of the b wave in the ERG obtained in response to sound + weak light (curves 40, 41, 88 and 89) are greater than the control recordings in response to the weak light alone (curves 38, 39, 83, 84, 90 and 91), which were obtained both before and after testing the conditioned reflex; here, if we compare curves 38 and 39 with curves 40 and 41 obtained after 164 reinforcements of the conditioned reflex, the difference amounts to approximately 25 μv, but when comparing curves 83-84-86 (weak light without sound) and curves 88-89 (weak light + sound) after 173 reinforcements, the difference now amounts to as much as 60-110 μv; again, when we compare curves 88-89 with the control curves 90 and 91 (experiment without sound, and weak light used alone) it amounts to about 50 μv. Other ERG parameters, such as the latent period of the b wave, time to attain the maximum and total duration, all showed less change.

A more detailed analysis of the different experiments showed that the sound does not immediately acquire the significance of a signal. Only after 50-60 coincidences was it possible to observe its conditioned reflex action and at this stage it was still weakly shown. The effect was very much better shown after 100-125 combinations, and the reflex was found to become extinguished very rapidly, and as a rule, on the following day, it either did not appear or else was shown very weakly. This feature of the formation of the conditioned reflex as affecting the ERG differs sharply from the behavior of sensory conditioned reflexes elaborated in connection with vision, where conditioned reflexes are elaborated very rapidly, after 2-5 coincidences, and are maintained for a long time (A. I. Bogoslovskii, E. N. Semenovskaya, R. B. Zaretskaya, and others).

While inducing conditioned reflex changes in the ERG, we incidentally observed similar alterations in the EEG. As would be expected, these developed considerably earlier than the corresponding ERG changes; the α-rhythm ceased in response to the sound somewhat before the light stimulation was given.

In this connection we investigated the possibility of an orienting reaction (arrest of α-rhythm by sound); the reaction was rapidly extinguished even in the first experiments and had no effect on the conditioned reflex extinction of the α-rhythm.

In the experiment performed on 10/7/1957, after the first 9 reinforcements of the conditioned reflex we observed no extinction of the α-rhythm to sound before the light stimulus was presented (see Fig. 3, trace 22), but in this same experiment (trace 36) after 36 reinforcements, before the strong light stimulus was given and at the moment of the sound stimulus, a well-marked depression of the α-rhythm was observed, showing that the sound stimulus was acting as a signal for the light. In the experiment performed on 16/7/1957 (see Fig. 3, trace 14), after 70 reinforcements of the conditioned reflex, this anticipatory arrest of the α-rhythm was still more pronounced: it began about 1 second before the strong light stimulus, 14 seconds after the sound had been turned on.

Our experiments are provisional, and are being continued. We think it worth while to publish some of them, as these observations are of importance in determining the part played by the central nervous system in the development of the human ERG and in connection with the problem of the interaction of central and peripheral processes in the visual analyser as a whole.

SUMMARY

The conditioned reflex changes were formed in the human electroretinogram. The ERG was registered with the aid of an active electrode soldered into the contact lens. The EEG of both occipital regions was registered simultaneously. Light (which gave about 600 lux illumination on the pupil) served as an unconditioned stimulus. Sound served as conditioned stimulus (60 decibel over the threshold of hearing, 250 cycles per sec) which had no effect by itself on the ERG. Numerous combinations of this sound with the light stimulus brought about the development of conditioned reflex changes in the ERG. This was manifested by the fact that in response to the mild light stimulation, preceded by sound, the amplitude of the electroretinographic wave "b" became higher than in response to the weak stimulation by light alone. In both cases the light which was employed as a stimulus gave an illumination on the pupil equal to about 80 lux and the wave "b" the amplitude of which was much lower than in light stimulus with 600 lux. The conditioned reflex changes were simultaneously observed in the EEG.

LITERATURE CITED

[1] R. B. Zaretskaya, in book: Collection of Information on Methods and Materials*, Helmholtz Scientific Research Institute of Ophthalmology, Moscow, No. 4, 48-55 (1956).

[2] A. V. Zonova, in book: Problems of Physiological Optics*, Moscow – Leningrad, No. 12, 367-376 (1958).

[3] J. Vaný sek, Csl. opthal. t 12, N. 4, 233-238 (1956).

* In Russian.

THE EFFECT OF SULFHYDRIL GROUPS ON VERATRINE SENSITIZATION OF MUSCLE TO THE ACTION OF POTASSIUM

Kh. S. Koshtoyants and L. S. Bunkina

From the Department of Animal Physiology, M. V. Lomonosov Order of V. I. Lenin Moscow State University

(Received April 10, 1958. Presented by Active Member of the AMN SSSR V. N. Chernigovskii)

Bacq [6] has published reports which show that veratrine causes a marked increase in the sensitivity of the striped muscle of amphibia to potassium ions. This interesting phenomenon has been subsequently confirmed for a number of substances which are nowadays classified as belonging to the alkaloids of the Veratrum group [7].

P. E. Dyablova [1] has shown that the sensitizing action of veratrine is not shown if the concentration of calcium ions in the Ringer solution is increased, and that there are chemical agents which can depress the sensitivity of muscles to calcium (e.g. novocain). Kh. S. Koshtoyants [2], in his experiments, has shown that the sensitivity of invertebrate (mollusc) smooth muscle to potassium is markedly increased by preliminary treatment with acetylcholine.

In the present article, we report the results of experiments carried out to reveal the role of the sulfhydril groups of protein complexes in the sensitizing action of veratrine.

The experiments represent a follow-up of the first experiment in this direction, which consisted of special investigations, showing that the typical picture of the time course of the contraction and the relaxation of veratrine treated muscle, which occur in response to the action of an electrical stimulus, do not take place when the sulfhydril groups are blocked, but reappear when these groups are reformed (L. S. Bunkina's experiments).

Also, in our laboratory, we have repeatedly demonstrated the dependence of the sensitivity of various muscles as well as chemoreceptors on the condition of the sulfhydril groups [3, 5]. It was shown in special experiments, that the increase in the sensitivity of denervated muscles to chemical stimulators also depends on changes in the concentration of the free sulfhydril groups which occurs at a definite time after the denervation [4]. From among the results reported, we must note that Sevag [8], in his theory of allergy, especially stresses the fact that the formation of sulfhydril and disulfide groups, by interacting with the tissue proteins and foreign substances, forms the basis of the increased sensitivity.

METHODS

The experiments were carried out on the rectus abdominis muscle of the frog. As is well known, when potassium chloride acts on this muscle, a contraction occurs which passes off after washing the solution away with Ringer. In order to induce veratrine sensitization of the muscle to the action of potassium we used a concentration of veratrine of $1 \cdot 10^{-6}$. To block the sulfhydril groups, we used a solution of cadmium chloride in a concentration of $1 \cdot 10^{-3}$; to regenerate the sulfhydril groups, a 1 in $5 \cdot 10^{-2}$ solution of unithiole was used.

The experiments were carried out simultaneously on the two recti muscles of a single frog; one muscle was used for the experiment, and the other as control. Both muscles were first treated with the same concentration

of potassium chloride, which gave about the same muscular contraction, and after this one muscle was treated with a solution of veratrine (control), and the other was treated simultaneously with solution of veratrine and cadmium chloride. The time of action of the solutions in both control and experimental muscles was 10 minutes.

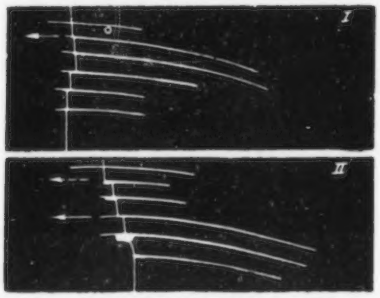


Fig. 1. Veratrine sensitization of the action of potassium (I); absence of veratrine sensitization of potassium action on blocking sulfhydril groups with cadmium chloride (II, first arrow) and subsequent restoration of veratrine sensitization of the action of potassium after regeneration of the sulfhydril groups by unithiole (II, second arrow), in both cases (I and II) the first contraction represents the response of the muscle before treatment with veratrine

RESULTS

In the control, as was to be expected, there was a sharp increase in the effect of potassium in the presence of veratrine, i.e. there was veratrine sensitization (Fig. 1). In this case, when the veratrine acted on the muscle at the same time as the cadmium chloride, the sensitivity of the muscle to the same concentration of potassium chloride was markedly reduced, or remained unchanged, i.e. there was no veratrine sensitization (Fig. 2, first arrow).

If the sulfhydril preparation unithiole acts for 10 minutes on a veratrinized muscle which has been treated with cadmium chloride, then for a given concentration of potassium chloride, there is a considerable increase in the contraction (Fig. II, second arrow), i.e. the veratrine sensitization has been restored. The restoration by means of the sulfhydril preparation unithiole of the veratrine sensitization destroyed by cadmium chloride indicates the part played by the sulfhydril groups in the sensitizing effect of veratrine. Consequently, sensitization to the effect of potassium ions on a muscle does not take place if the muscle is treated simultaneously with solutions of veratrine and cadmium chloride. Veratrine sensitization of a muscle treated simultaneously with veratrine and cadmium chloride can be restored by treating the muscle with a solution of the sulfhydril preparation unithiole.

The results give reason to suppose that the sulfhydril groups of proteins play a particular part in veratrine sensitization. This action is associated with the part played by the sulfhydril groups in the integration of the protein structures.

SUMMARY

If the muscle is simultaneously treated with veratrine and cadmium chloride solutions there is no veratrine sensitization effect of potassium ion action on the muscle. This sensitization may be reestablished after the treatment of the muscle with unithiol, the sulfhydryl preparation solution.

A conclusion is drawn that the sulfhydryl group of protein bodies play a definite role in the sensitization of potassium ions on the muscles with veratrine. This is connected with the special part played by the sulfhydryl groups in the integration of the protein structures.

LITERATURE CITED

[1] P. E. Dyablova, Fiziol. zhurn. SSSR, volume 32, No. 5, 647-650 (1946).

[2] Kh. S. Koshtoyants, Byull. éksptl. biol. i med., volume 2, issue 3, 185-187 (1936).

[3] Kh. S. Koshtoyants, Protein Bodies, Metabolism and Nervous Regulation*, Moscow (1951).

[4] M. F. Popova, Fiziol. zhurn. SSSR, volume 42, No. 11, 977-980 (1956).

[5] G. Yu. Yur'eva, Biofizika, volume 2, issue 6, 665-669 (1957).

[6] Z. M. Bacq, Compt. rend., Soc. Biol. v. 130, 1369-1371 (1939).

[7] R. Goutier, Brit. J. Pharmacol. v. 5, 33-36 (1950).

[8] M. G. Sevag, Ann. Allergy v. 14, 233-246 (1956).

* In Russian.

EFFECT OF TEMPERATURE ON THE EXCITABILITY OF PIGEON MUSCLE

I. P. Suzdal'skaya

From the Laboratory of Cellular Physiology (Head – Active Member of the AMN SSSR Professor D. N. Nasonov)* Physiological Institute (Director – Professor N. V. Golikov) Leningrad State University

(Received April 26, 1956. Presented by Active Member of the AMN SSSR D. N. Nasonov) *

In previous papers dealing with investigations of the effect of temperature on isolated frog and turtle nerves [2, 3], it was shown that the constants a and b in Gorveg's** formula [8] vary in opposite directions with temperature changes of 10° made in the temperature range from 5 to 30° C. On cooling, the rheobase of the tissue (constant b) is reduced, and the excitibility to stimuli of long duration increases; at the same time the threshhold of short-term excitability (constant a) increases, i.e. the excitability to short stimuli falls. As a result, the strength-duration curves of cooled and warmed nerves intersect, and the excitability to stimuli in the area where the curves intersect remains constant over a certain temperature range. (The nerves of winter frogs form an exception, and, unlike those of summer frogs, the strength-duration curves for the heated and cooled nerves do not intersect.)

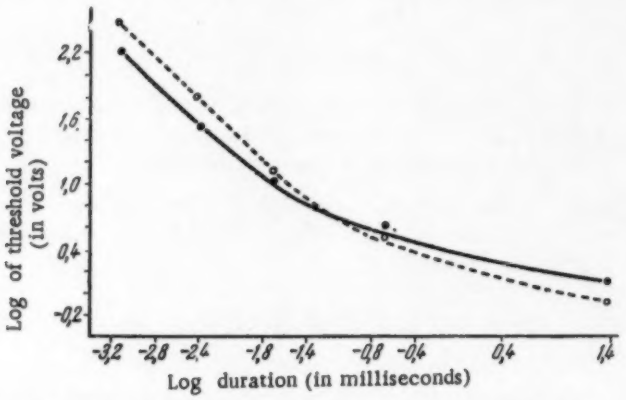


Fig. 1. Logarithmic curves of strength – duration for the m. flexor metacarpi radialis of the pigeon wing at 39°C (continuous line) and at 28.5°C (dotted line).

* Deceased.

** D. N. Nasonov and D. L. Rosental' [1] showed that the constants a and b of Gorveg's formula $V = (a/t) + b$ can be used to describe tissue excitability if the index of $t = I$. The constant a determines the excitability to very short discharges. When t is very small, the value of a/t in the formula $V = (a/t) + b$ becomes so large that the value of b can be neglected; then $V = (a/t)$ or $a = t \cdot V$. The constant b determines the sensitivity to stimuli of long duration, and is independent of the duration, because when t becomes very large, the value of a/t in the formula $V = (a/t) + b$ becomes negligeable, and then $V = b$.

TABLE 1

Changes in the Excitability Constants for the m. flexor metacarpi radialis of the Pigeon Wing on Changing the Temperature from 39 to 29°C, and from 29 to 39°C (mean values from 7 experiments and standard deviations)

From 39 to 29°C				From 29 to 39°C			
a		b		a		b	
initial value (mv/msec)	after cooling (% of initial value)	initial value (volts)	after cooling (% of initial value)	initial value (mv/msec)	after heating (% of initial value)	initial value (volts)	after heating (% of initial value)
104,1	+48,7±13,5	1,03	−32,7±3,3	157	−35,0±5,3	0,86	+34,0±3,8

Key: a) threshold of short-term excitability; b) threshold of long-term excitability (rheobase).

TABLE 2

Variation in the Excitability Constants of the m. flexor metacarpi radialis of the Pigeon Wing on Changing the Temperature from 30 to 20°C and from 20 to 30°C (mean values of 7 experiments and standard deviations)

From 30 to 20°C				From 30 to 30°C			
a		b		a		b	
initial value (mv/msec)	after cooling (% of initial value)	initial value (volts)	after cooling (% of initial value)	initial value (mv/msec)	after heating (% of initial value)	initial value (volts)	after heating (% of initial value)
103,0	+212,0±88	0,91	−28,0±4,2	370,0	−58,0±10,2	0,73	+38,0±7,2

Key: symbols as in Table 1.

TABLE 3

Variation of the Excitability Constants of the m. flexor metacarpi radialis of the Pigeon Wing on Changing the Temperature from 20 to 10-8°C and from 10 to 20°C (mean values from 10 experiments and standard deviations)

From 20 to 10°C				From 10 to 20°C			
a		b		a		b	
initial value (mv/msec)	after cooling (% of initial value)	initial value (volts)	after cooling (% of initial value)	initial value (mv/msec)	after heating (% of initial value)	initial value (volts)	after heating (% of initial value)
385,0	+208,0±52	1,11	+51,0±17,6	1486,0	−64,0±6,2	1,86	−36,0±9,6

Key: symbols as in Table 1.

From this it may be concluded that the nerves of cold-blooded animals have the power to stabilize their excitability in face of temperature changes, and that this constancy is maintained over the range of stimuli of moderate length, a length which is approximately equal to that of physiological stimuli.

There are some differences between cold- and warm-blooded animals with respect to the dependence of the excitability of their nerves on temperature. The nerves of the former are able to regulate the excitability to voltages of a certain length over a wide range of temperatures [2, 3], while in the latter, the regulation of the excitability is effective only over a narrow temperature range – from 40 to 35°C [5].

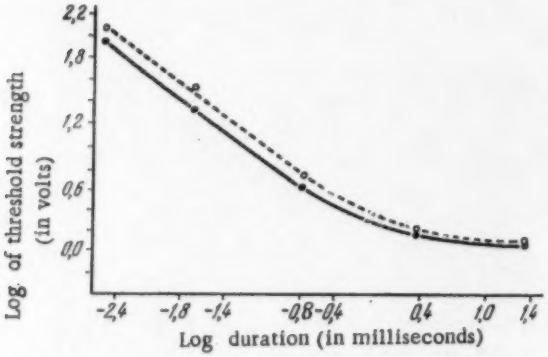


Fig. 2. Logarithmic curves of strength – duration for the m. flexor metacarpi radialis of the pigeon wing at 20°C (continuous line) and at 10°C (dotted line).

A study of the excitability of the muscles of warm-blooded animals (rats) at different temperatures [4] has shown that on cooling from 20 to 10°C, i.e. over a range of low temperatures, the excitability of the muscles both to short (a) and to long (b) stimuli is reduced, and that on heating to 20°C it increases. However, on cooling or heating at higher temperatures – from 20 to 30°C and from 30 to 40°C – the excitability of rat muscle changes in the same way as does the excitability of both nerve and muscle in cold-blooded animals [3, 5, 7], i.e. the constants a and b change in opposite directions. Cooling causes an increase in excitability to stimuli of long duration (reduction of constant b) and a reduction of excitability to short-acting stimuli (increase of constant a). On heating, the reverse effect obtains: the excitability to stimuli of long duration is reduced, while that to short stimuli is increased.

On account of these results we have undertaken a further set of experiments on the muscle of warm-blooded animals, but we have used another class – that of the birds in order to discover whether the feature which we found previously – the dependence of the excitability of rat muscle on temperature, is also true for warm-blooded animals in general, or whether it is only a property of one particular species. The animals which we used for these experiments were pigeons.

METHOD

The muscle employed was the m. flexor metacarpi radialis, which was particularly useful for the task in hand. The muscles were isolated, bathed in Ringer's solution, and placed on electrodes separated by a distance of 1.5 cm in a chamber through which flowed vaseline oil whose temperature could be changed by 10°C in 3-5 minutes; the temperature could be kept constant to an accuracy of 1°C. Stimulation of the preparation was effected by condenser discharges of different durations. The effect of the stimulation was recorded by the contraction of the muscle. In some experiments the whole strength-duration curve of the muscle was plotted; in others, in order not to damage the preparation, the measurements were confined to the constant b – the rheobase measured in volts, and the constant a – the short-term excitability, where a was found, as had been proposed by D. N. Nasonov and D. L. Rozental' [1], by multiplying the potential in millivolts required to cause a muscular contraction by the corresponding duration in milliseconds (mV · msecs). The results were considered reliable if the difference between the mean values, expressed as a percentage, was greater than three times the root mean square error.

RESULTS

Altogether 3 sets of experiments were carried out over temperature ranges of 29-39°C, 20-30°C, and 20-10-8°C. There were 7 experiments in each of the first and second groups, since the change of excitability with temperature was very much the same for the two temperature ranges. In the third group, there were 10 experiments. On cooling the m. flexor metacarpi radialis from 39 to 29°C, the excitability to brief stimuli fell on average by 48.7% (the threshold value represented by the constant a increases), and the excitability to stimuli of long duration increased by 32.7% (the threshold corresponding to the constant b, or rheobase, decreases). Warming

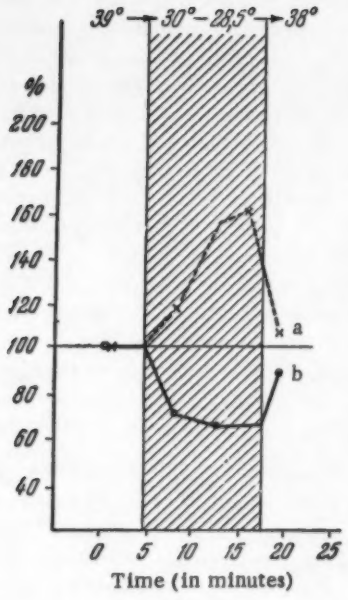


Fig. 3. Changes in the constant for short-term (a) and long-term (b) excitability of the m. flexor metacarpi radialis of the pigeon wing (in % of initial value) on cooling the muscle from 30 to 20-18°C and subsequently reheating to 30°C.

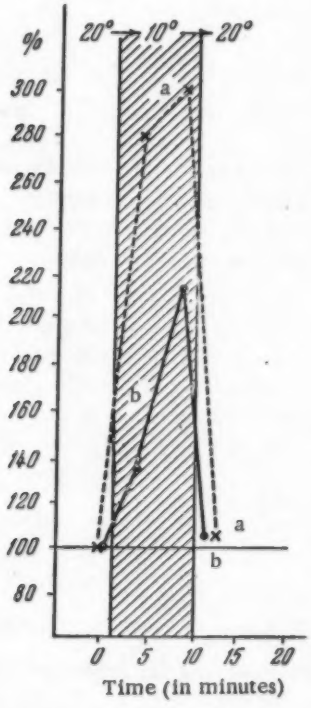


Fig. 4. Changes in the constant for short-term (a) and long-term (b) excitability of the m. flexor metacarpi radialis of the pigeon wing (in % of initial value) on cooling the muscle from 20 to 10°C and subsequently reheating to 20°C.

the muscle caused a reversal of this result: the excitability to brief stimuli increased by 35%, and that to long stimuli fell by 34% (Table 1).

A logarithmic plot of the strength-duration curve of one such experiment is shown in Fig. 1. Here the strength – duration curves taken at 39 and 29°C intersect at a value of 0.05 milliseconds. Fig. 2 shows changes in the constants a and b found during the same experiment, and here, the values obtained after heating or cooling are expressed as a percentage of their original values. It is clearly seen from the figure that on cooling, the two constants changed in opposite directions, and that on warming they almost returned to their initial values.

In the second set of experiments, a study was made of the excitability of the m. flexor metacarpi radialis on cooling from 30 to 20°C, and then reheating to 30°C. As can be seen from Table 2, on lowering the temperature from 30 to 20°C, the excitability changed in the same direction as was observed in the first set of experiments: on cooling, the rheobase was reduced by 28% (the excitability increased), while on heating, it increased by 38% (the excitability fell); at the same time, the excitability to brief stimuli was increased by 58% on heating to 30°C, and on cooling to 20°C it dropped sharply – on average by 212%. Here the standard deviation is very great, since the fall in the excitability in these experiments varies from 60 to 700%.

Thus, in the first and second sets of experiments, the excitability constants (a and b) of the pigeon muscle change in opposite directions, and are temperature-dependent both for long stimuli lasting from 0.20 to 24 milliseconds, as well as for short stimuli of from 0.02 to 0.008 milliseconds; the excitability to short stimuli (a) changes several times more than does the rheobase (b). However, in all experiments of the first two groups, there is a region in the strength-duration curves in which the curves of both the heated and cooled muscles intersect, i.e. for a certain intermediate duration of electrical stimulus, the excitability is either independent of the temperature or depends very little on it.

In the third set of experiments, we

tried to reduce the temperature of the muscle still further, lowering it from 20 to 10°C and sometimes as far as 8°C (on cooling below 8°C the muscle excitability failed), after which the temperature was returned to the initial value. It was found that on cooling, the excitability to brief stimuli falls, on average, by 208%, i. e. the same effect is observed to occur on changing temperature in the experiments described above. However, there is now the difference that the excitability to stimuli of long duration is also lowered by 51%. On heating, on the contrary, the excitability to short and to long stimuli is increased, so that both constants a and b are reduced. As can be seen from Table 3, the results of this set of experiments are statistically significant. Fig. 2 shows strength - duration curves from one of these experiments. The curves do not intersect, and any point on the curve falls in a region where the excitability is related to temperature; the relationship is different in the two cases – the excitability to short stimuli being greater than that to stimuli of long duration. Fig. 4 shows the changes in constants a and b from one of the experiments of this set, where the excitability of muscles to short and to long stimuli falls on cooling, and almost returns to its initial value on heating. The difference between the changes of the constants a and b in pigeon muscles on changing from one temperature range to another, can be very clearly seen by comparing Figs. 1 and 2 with Figs. 3 and 4. In the first case, i. e. on changing the temperature from 39 to 20°C, the constants a and b change in opposite directions; in the latter case, when the experiments are carried out in a low temperature region, from 20 to 10°C, both constants change in the same way.

The results we have described allow us to conclude that temperature changes in the temperature range from 20 to 40°C causes little change in excitability for stimuli of moderate length, but that for either long or short stimuli, the excitability changes in opposite directions. On changing the temperature from 20 to 10°C, the excitability of pigeon muscle to stimuli of any length falls on cooling and increases on heating. Similar results were obtained for both tonic and tetanic muscles of the rat [6]. Thus, no essential differences in the excitability reactions of muscles were found to differentiate homothermic from poikilothermic animals [5, 6, 8]. The only feature distinguishing the two groups of animals is the particular temperature range over which the strength-duration curves intersect, the intersection showing that there is a region over which the excitability to stimuli of moderate duration is constant. For muscles of warm-blooded animals, this temperature ranges from 40 to 20°C, while for the muscles of cold-blooded animals, it extends from 25 to 5°C. In other words, for warm-blooded animals in general, cell regulation of excitability in response to temperature change does occur, but over a different temperature range than for cold-blooded animals.

SUMMARY

The author studied the effect of cooling and warming of pigeon's m. flexor metacarpi radialis wing on its excitability to prolonged and short electric stimuli, and on the voltage-time curve. In cooling the muscle from 20° C to 10° and 8° C and its subsequent warming to 20° C there is no intersection of the voltage-time curves. Excitability to stimuli of any duration depends on temperature: it decreases with cooling and increases with warming of the muscle. Cooling of the muscle from 40° C to 30° C and from 30° C to 20° C increases the excitability to prolonged stimuli and decreases it to short ones. In warming (within the limits of the temperatures mentioned above) the excitability to prolonged stimuli is decreased and to short is increased.

The voltage-time curves cross in cooled and warmed muscles. Therefore, the excitability of the muscles to currents of certain medium duration (approximately corresponding to the duration of the physiological impulse), shows an insignificant change at various temperatures. Consequently, there is a cellular adjustment existing in the muscles of both warm- and cold-blooded animals, controlling the excitability of the muscles and protecting them from sharp changes at various temperatures.

LITERATURE CITED

[1] D. N. Nasonov and D. L. Rozental', Fiziol. zhurn. SSSR, 39, No. 4, 405 (1953).

[2] D. N. Nasonov and I. P. Suzdal'skaya, Fiziol. zhurn. SSSR, 42, No. 4, 415 (1956).

[3] D. N. Nasonov and I. P. Suzdal'skaya, Zhurn. Biofizika, volume 1, issue 4, 306 (1956).

[4] D. N. Nasonov and I. P. Suzdal'skaya, Fiziol. zhurn. SSSR, 44, No. 11, 1034-1039 (1958).

[5] I. P. Suzdal'skaya, Fiziol. zhurn. SSSR, 43, No. 1, 80 (1957).

[6] I. P. Suzdal'skaya, Fiziol. zhurn. SSSR, 43, No. 5, 449 (1957).

[7] I. P. Suzdal'skaya and M. B. Kiro, Byull. éksptl. biol. i med. volume 43, No. 5, 28-31 (1957).

[8] G. Hoorweg, Pflug. Arch. Bd. 52, 87 (1892).

VASOMOTOR REGULATORY MECHANISMS

COMMUNICATION III. REFLEXES INVOLVING EFFERENT PATHWAYS TO THE VESSELS OF THE LIMBS AND AFFERENTS FROM SOMATIC NERVES

V. M. Khayutin and V. L. Tsaturov

From the Experimental Laboratory (Head – Candidate of Medical Sciences V. M. Khayutin) Institute of Normal and Pathological Physiology (Director – Active Member of the AMN SSSR V. N. Chernigovskii) AMN SSSR, Moscow

(Received April 24, 1958. Presented by Active Member of an AMN SSSR V. N. Chernigovskii)

In a previous communication [7]. it was shown that electrical stimulation of afferent fibers of somatic nerves initiates vasomotor reflexes affecting the vessels of the extremities, and that the effects are closely related to the strength of the stimulus. Comparatively weak stimuli cause a reflex dilatation, which we will refer to as primary. On increasing the strength of the stimulus, the effect changes to a contraction of the blood vessels. Still stronger stimuli cause the vasoconstriction to give place to a secondary vasodilatation.

The last phenomenon outwardly resembles the "perversion" of spinal motor reflexes, described by Sherrington and Sowton [1, 16, 17]. In our experiments, a relatively weak stimulation of the afferent fibers of the ipsilateral limb (in case of the extensor reflex) or of the contralateral limb (in case of the flexor reflex) brought about contraction of the corresponding muscles. As the stimulus increased, the contraction increased a certain amount, after which it invariably changed into a relaxation of the muscle, i.e. a typical reciprocal inhibition took place.

At a certain strength of more or less prolonged stimulation, the flexor or extensor reflex takes place biphasically; the initial contraction of the muscle changes into a relaxation. The regional vasoconstrictor reflex takes place in precisely this way, changing over during the stimulation into a vasodilator effect.

There can be no doubt that the "perversion" of motor reflexes is due to the development of central inhibition. Whatever view of the mechanism of the latter is held, the relaxation of muscle which replaces the contraction can only occur through the inhibitory effect supervening in the motor neurones of skeletal muscle.

This is not the case for the relaxation of the smooth muscles of the blood vessels. Theoretically, the reflex dilatation of the vessels might take place in the following ways: first, by central inhibition of vasoconstrictor tone continuously maintained by sympathetic adrenergic fibers [8 and others], secondly, by stimulation of vasodilator fibers originating in the dorsal roots [10, 3, 9 and others] or, by the excitation of sympathetic vasodilator cholinergic fibers [12, 14, 15, 18]. It is only possible to talk of a "perversion" of a vasomotor reflex if the secondary vasodilatation is brought about by the first of the ways just enumerated, i.e. as a result of central inhibition of sympathetic vasoconstrictor tone. The present work consists of an investigation of this problem.

METHOD

The experiments were performed on cats, and a continuous recording was made of the resistance of the blood vessels to fluid flow while perfusing the limbs at a stabilized minute volume of blood. The principle of the method

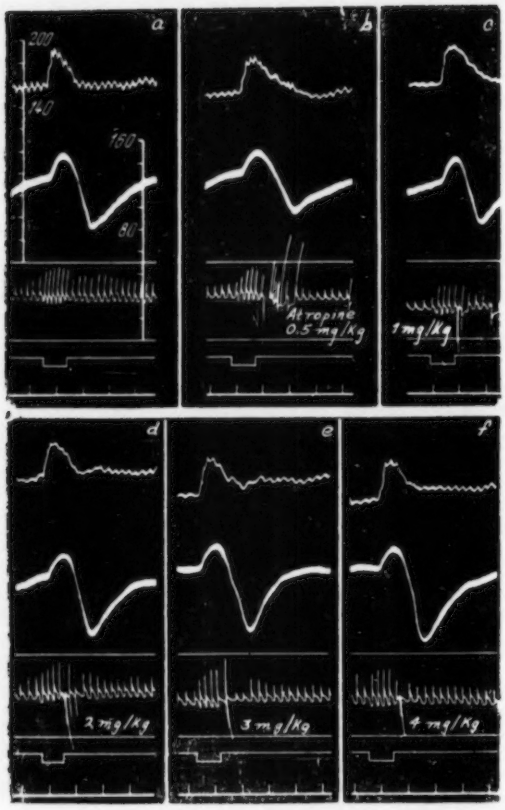


Fig. 1. Absence of any changes in secondary regional vasodilation after injecting atropine. Stimulation of the right peroneal nerve (alternating current, 50 c/s, 4 v). Perfusion of left hind limb: a) before injection atropine; b) after injecting 0.5 mg/kg; c) 1 mg/kg; d) 2 mg/kg; e) 3 mg/kg; f) 4 mg/kg of atropine injected into the jugular vein. Curves, from above downwards: arterial pressure in carotid artery, perfusion pressure in left femoral artery, zero line of arterial pressure, respiration, zero line of perfusion pressure, applied stimuli, time markings — 30 seconds.

and the apparatus have been described previously [4, 5, 6]. The animals were anesthetized with urathane (0.5 g/kg) and chloralose (0.03 g/kg). Coagulation of the blood was prevented by heparin (7.5-12.5 mg/kg). The arterial pressure and the respiration were recorded by the usual methods. Usually the central end of the cut nerve was stimulated, and the nerve used was usually the sciatic or the peroneal nerve of the leg opposite the perfused one, or else the peroneal nerve cut at the level of the malleolus of the perfused limb; a 50 cycle alternating current was used, the voltage on the electrodes being indicated by a voltmeter. In some cases, a square wave stimulator was used having independent frequency and amplitude controls and a fixed duration of 1 millisecond. In all, 21 experiments were performed.

RESULTS AND DISCUSSION

The change-over of the limbs to a dilatation, occurring during the stimulation of the afferent fibers, was found by Binet and Burstein [11] in experiments on dogs, and was also found by us in cats [7]. In both animals, the blood vessels of skeletal muscle are innervated by sympathetic vasodilator fibers. As is well known, these fibers are cholinergic: vasodilatation induced by electrical stimulation of the efferent pathways originating in the motor cortex and ending in the abdominal sympathetic chain is eliminated by atropine in doses of 0.1-0.3 mg/kg [12, 14, 15, 18].

Also, atropine has no effect on reflex dilatation of the limb vessels occurring in response to stimulation of the central end of the brachial nerve by weak low-frequency pulses (we have previously described this kind of vasodilatation [7] as "(primary regional vasodilalation);" it has no effect either on the vasodilatation induced by stimulation of the mechanoreceptors of the carotid sinus and aortic arch [12, 14]. Consequently, the sympathetic dilator fibers can take no part in these reflexes.

As can be seen from the kymograms shown in Fig. 1, intravenous injection of atropine in doses up to 4 mg/kg has no effect on the secondary regional vasodilatation. This dose is 13-14 times greater than that necessary for complete suppression of the dilatation of the vessels of the muscles of the hind limb induced by direct stimulation of the sympathetic cholinergic vasodilator fibers. Similar results were obtained in two further experiments.

It is therefore clear that the secondary regional vasodilatation is mediated by sympathetic vasodilators, and that these fibers (at any rate in an acute experiment) are not involved in the vasomotor reflexes mediated by the somatic nerves.

Bayliss [10], L. Fofanov [3], M. A. Chalusov [9], and many other authors have maintained that the vessels of sympathectomized limbs retain their ability to dilate in response to stimulation of the aortic nerve or of the central end of the vagus. In experiments of this type, the only pathway for the transmission of reflex effects to these vessels is via the fibers of the dorsal roots. It therefore seemed possible that antidromic transmission of impulses

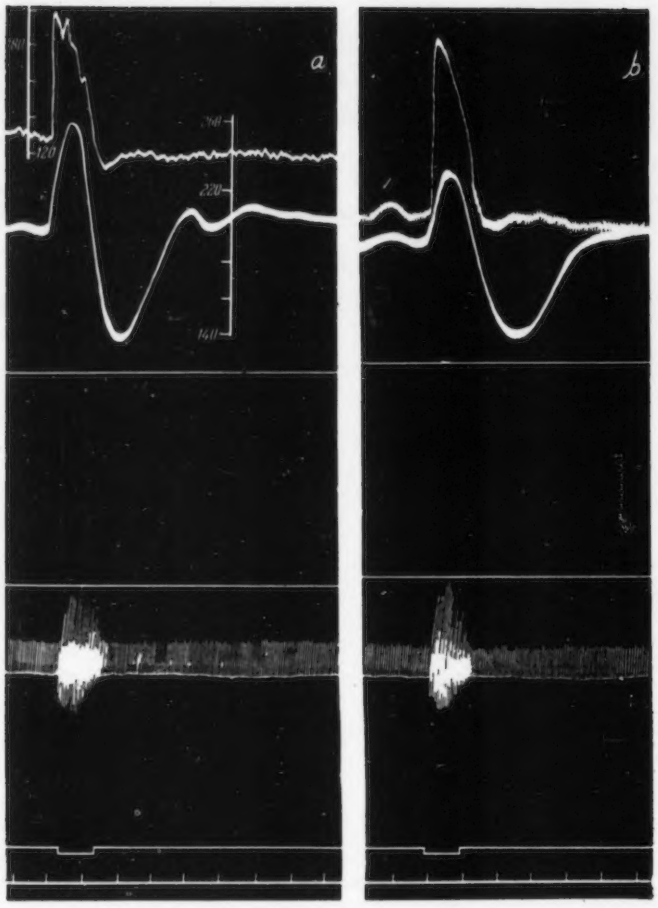


Fig. 2. Secondary regional vasodilatation before (a) and after (b) division of the posterior roots of the perfused limb. Stimulation of the right peroneal nerve (alternating current, 50 c/s, 2 v). Perfusion of left hind limb. Sequence of traces as in Fig. 1.

from the central nervous system to the vessels of the limbs along the afferent fibers might occur [10]. The existence of a reflex antidromic mechanism of vasodilatation as postulated by Bayliss has been much discussed. Many different hypothesis have been advanced to explain this paradoxical phenomenon [2]. However, recent researches have shown that there is no need for such hypotheses, since Bayliss's phenomenon is not neurogenic.

In their investigations, Folkow, Ström and Uvnäs [13], and Frumin, Ngai and Wang [14] have given a detailed analysis of the artifacts due to using plethysmography and many other methods in the measurement of the blood supply to organs – artifacts which have led to the idea of antidromic vasodilatation as part of a reflex.

Once they had eliminated the possibility of hemodynamic influences, these investigators found no vascular response in sympathectomized limbs on stimulating the carotid sinus, afferent fibers of the vagus, or the so-called "depressor zone" of the bulbar vasomotor center. Although their results are completely convincing, the results of their experiments cannot automatically be assumed to be due to secondary regional vasodilatation.

We have studied systematic and regional reflexes before and after division of the posterior roots of the lumbosacral section of the spinal cord on the same side as the perfused limb. Twelve such experiments were carried out. The spinal canal was opened, using a special milling cutter, while carefully preserving the spinal cord and dorsal roots from damage. After 1½-2 hours from the time of opening the canal and cutting the dura, an injection of

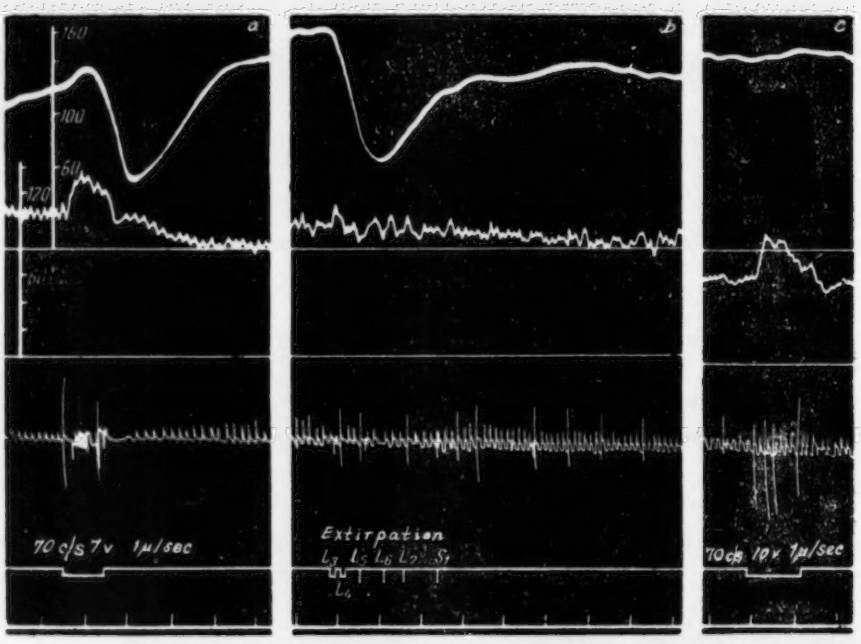


Fig. 3. Disappearance of regional vasomotor reflex after unilateral lumbar sympathectomy (from L_3 to S_1); a,b) stimulation of the right sciatic nerve (a – square pules, duration 1 millisecond, 70 c/s, 7 v, b – same,10 v); c) moment of sympathectomy. Perfusion of left hind limb.

heparin was given, and the main part of the experiment begun. After a few stimulations applied to the nerve of the contralateral limb, the posterior roots on the side of the perfused limb were divided, and reflex stimulation again applied. Typical results for this set of experiments are shown in Fig. 2, from which it can be seen that section of the posterior roots has no effect on the systemic or regional reflexes or, which was especially interesting to us, on secondary regional vasodilatation either.

The second observation, however, required confirmation. In some of the experiments, the secondary regional dilatation disappeared immediately after division of the posterior roots, and then gradually returned to its original value over a period of 20-30 minutes. The reason for this phenomenon is not at all clear. It could be due to stimulation of the posterior roots at the time of the division, or, as would appear more probable, to a reduction of the "background" afferent impulses. However that may be, secondary vasodilatation soon reappears. It follows therefore that the posterior roots cannot be the efferent pathway mediating this effect. Thus, there remains only one nervous mechanisms which could subserve this reaction, and that is central inhibition of sympathetic vasoconstrictor tone.

This conclusion was tested in 6 experiments with unilateral excision of the sympathetic ganglia from the 2nd-3rd lumbar to the 1st-2nd sacral segments. After opening the abdominal cavity, long ligatures were placed under the ganglia. The abdominal cavity was then sewn up, and after several "test" stimuli, the sympathetic ganglia were removed by pulling on the ligatures. After sympathectomy, it was not possible to elicit the regional reflexes by stimuli of any strength (Fig. 3).

In this way it can be seen that secondary regional vasodilatation results from reflex inhibition of tonic vasoconstrictor impulses. It therefore follows that the change-over from reflex regional vasoconstriction to vasodilatation resembles the "perversion" of spinal motor reflexes. More detailed results on the mechanism of "perversion" of regional vasomotor reflexes will be presented later.

SUMMARY

Secondary regional vasodilatation occurring in strong stimulation of the afferent fibers of somatic nerves was investigated in experiments on cats. The reflexes of the blood vessels of the posterior extremity were studied by the method of resistography. The exclusion of the sympathetic vasodilating fibers with atropine and the division of the posterior roots of the perfused extremity have no effect on secondary regional vasodilatation. The latter disappears after unilateral lumber sympathectomy. Thus, secondary regional dilatation is the sequence of the reflex inhibition of vasoconstricting fibers. The similarity of the mechanism of "perversion" of vasomotor and spinal motor reflexes was established.

LITERATURE CITED

[1] R. Krid and others, Reflex Activity of the Spinal Cord,* Moscow – Leningrad (1935).

[2] L. A. Orbeli, Lectures on the Physiology of the Nervous System,* Leningrad (1935).

[3] L. Fofanov, The Physiology of n. depressoris, * Kazan' (1908).

[4] V. M. Khayutin, Fiziol. zhurn. SSSR, 44, No. 7, 645 (1958).

[5] V. M. Khayutin, Byull. éksptl. biol. i med. 46, No. 10, 18 (1958).

[6] V. M. Khayutin, V. M. Danchikov and V. L. Isaturov, Byull. éksptl. biol. i med. 45, No. 2, 117 (1958).

[7] V. M. Khayutin and V. L. Tsaturov, Byull, éksptl. biol. i med. 47, No. 2, 17 (1959).

[8] I. F. Tsion, Course in Physiology,* St. Petersburg, 1873-1874, Vol. 1.

[9] M. A. Chalusov, Nevropatol. vestn. 16, No. 1, 132 (1909).

[10] W. M. Bayliss, The Vasomotor System, London (1923).

[11] L. Binet and M. Burstein, Comp. rend.Soc. Biol. v. 141, 771 (1947).

[12] B. Folkow and B. Uvnäs, Acta physiol. scandinav. v. 15, 389-400 (1948).

[13] B. Folkow, G. Ström and B. Uvnäs, Acta physiol. scandinan, v. 21, 145-155 (1950).

[14] M. J. Frumin, S. H. Ngai and S. C. Wang, Am. J. Physiol., v. 173,428-436 (1953).

[15] P. Lindgren, Acta physiol. scandinav. supp. 121 (1955).

[16] C. S. Sherrington and S. C. M. Sowton, Proc. Roy. Soc. Sec. B, v. 83, 435 (1911).

[17] C. S. Sherrington and S. C. M. Sowton, Proc. Roy. Soc. Sec. B, v. 84, 201 (1911).

[18] B. Uvnäs, Physiol. Rev. v. 34, 608-618 (1954).

* In Russian.

THE ROLE OF THE ADRENALS IN THE RESTORATION OF THE VITAL FUNCTIONS AFTER CLINICAL DEATH*

M. G. Kolpakov, M. G. Polyak and G. S. Yakobson

From the Department of Pathological Physiology (Head - Docent G. L. Lyuban) of the Novosibirsk Medical Institute (Director - Prof. G. D. Zalesskii)

(Received June 30, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

Resuscitation after clinical death depends primarily on restoration of the functions of the central nervous system, which is most sensitive to oxygen lack [9, 10].

Restoration of the disturbed central control determines the subsequent vital activities of the body and the normal functioning of its effector systems and organs. At the same time it must be borne in mind that disturbances of metabolism have an effect on the centers of the brain. Even in the period of restoration of the activity of the midbrain and of the appearance of bioelectrical activity in the cortex, glycolytic processes are still predominant over oxidative, and the blood contains an increased amount of organic acids [2, 11].

Incompletely oxidized products of metabolism and toxic substances formed in the tissues during oxygen starvation may have an unfavorable effect on the restoration of the functions of the brain. In this connection it is important to study the role of the adrenal glands in resuscitation, for as effectors of the second order [3], they play a direct part in the processes of metabolism, or, according to the findings of several workers, they bring about neutralization of the toxemic factors [1, 4, 15 and others].

With this in view, we studied resuscitation after clinical death in adrenalectomized animals, some of which, for replacement therapy, received cortisone and a special salt-rich diet [8]. Resuscitation of the adrenalectomized animal was first carried out in V.A. Negovskii's laboratory in order to discover the role of the adrenals in disorders of carbohydrate metabolism [2].

EXPERIMENTAL METHOD

The experiments were carried out on cats of both sexes. A one-stage bilateral adrenalectomy was performed under intratracheal ether anesthesia, with sterile precautions, through a retroperitoneal approach. The animals of series A were used in the experiment from 16-17 hours after adrenalectomy. Experiments on 10 animals showed that the length of survival of cats after one-stage bilateral adrenalectomy varied between wide limits – from 23 to 158 hours (average – 60 hours).

The experiments in series B were carried out on animals 4 days after adrenalectomy. For 3 days after the operation the animals received cortisone (10 mg/kg once daily). The last injection of the drug was given 24 hours before the experiment. The mineral balance and nutrition were maintained by the administration of salt solution and milk through a tube. The experiments in series C were performed 4 days after adrenalectomy. In the postoperative period the animals received large doses of cortisone (10 mg/kg twice a day), salt solution and milk. The last injection of cortisone was given just before the experiment. Clinical death was induced by bleeding. From 4-5 minutes after the last breath of the animal in a state of agony, resuscitation by V. A. Negovskii's method was commenced, without addition of glucose to the blood and, so far as possible, without adrenalin.

* The subject matter of this paper was presented at the conference of the Novosibirsk Medical Institute on April 10, 1958.

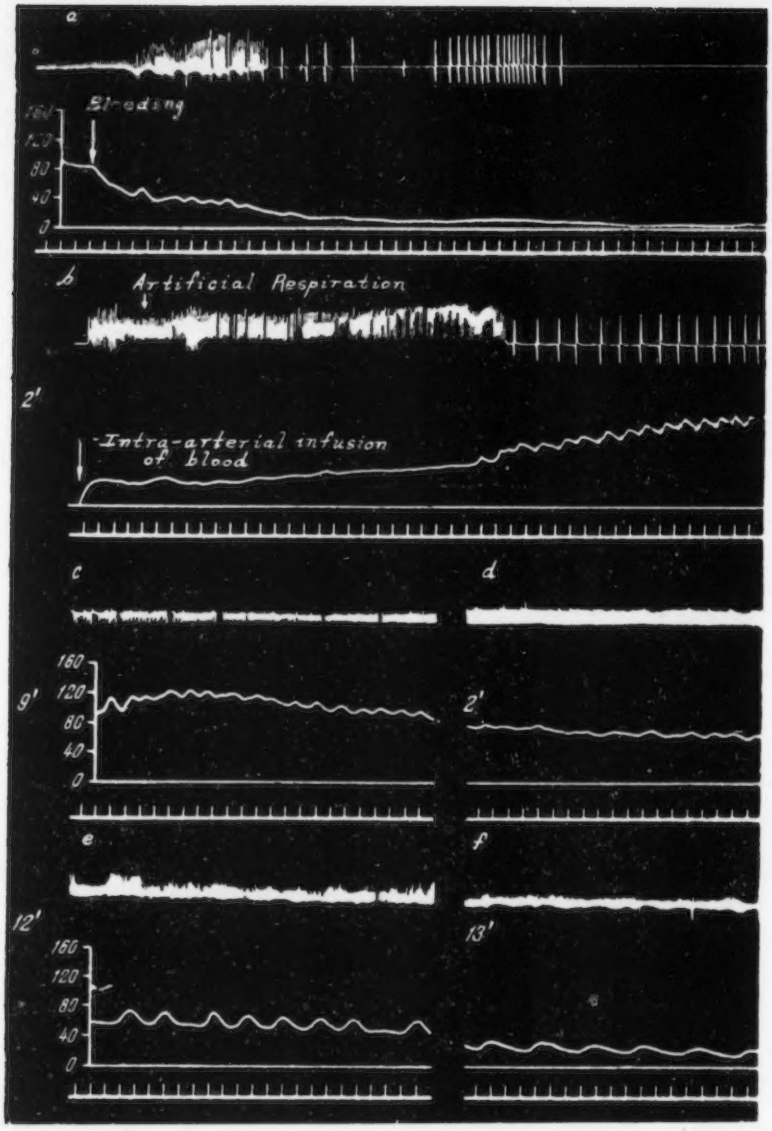


Fig. 1. Restoration of the vital functions after clinical death of a cat in an experiment dated March 30, 1958, using artificial respiration and intra-arterial infusion of blood.

a) Bleeding, onset of clinical death; b) resuscitation and period of independent agonal respiration; c) 9 minutes after restoration of spontaneous respiration; d) 11 minutes after restoration of spontaneous respiration; e)12 minutes after. Significance of the curves from above down: respiration, arterial pressure, zero line and time marker - 10 seconds.

EXPERIMENTAL RESULTS

The adrenalectomized cats (16-17 hours after adrenalectomy) could be brought out of the state of clinical death by means of artificial respiration and arterial blood transfusion in the majority of cases even without administration of adrenalin (Fig. 1).

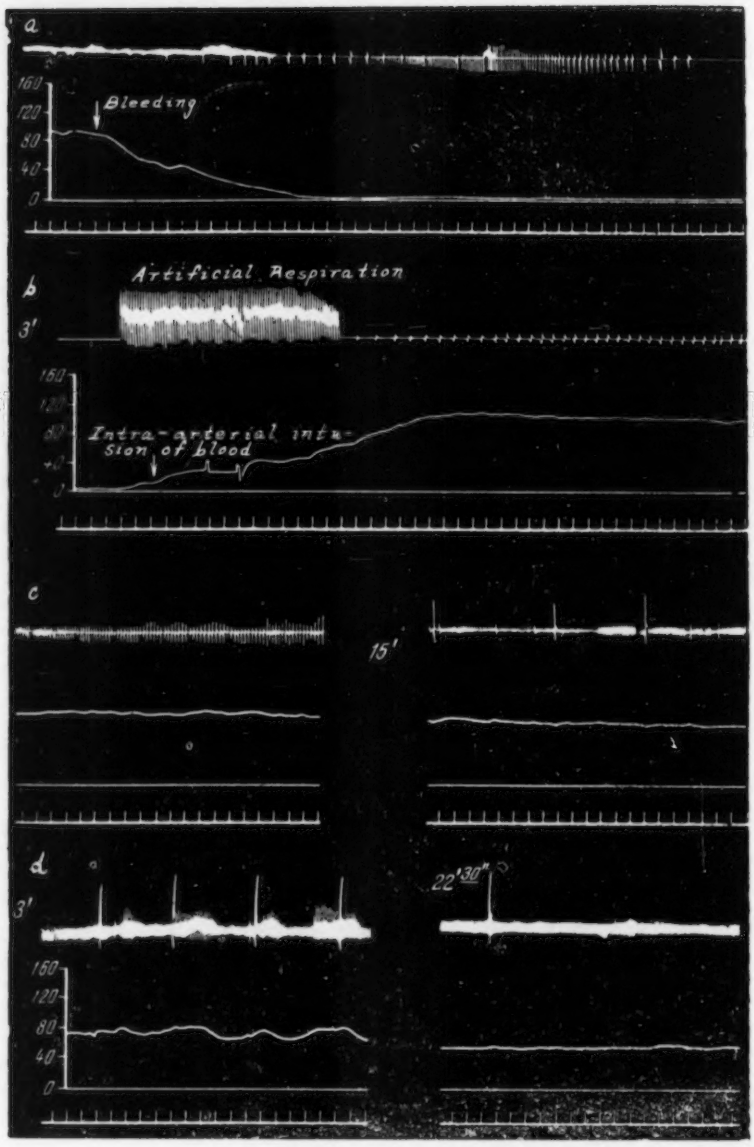


Fig. 2. Restoration of the vital functions after clinical death of a cat in an experiment dated April 11, 1958, in which cortisone was injected in the postoperative period. Legend as in Fig. 1.

During resuscitation the blood pressure and respiration were rapidly restored, but the subsequent normalization of the vital functions was much more difficult. The suggestion was made that the subsequent normalization of functions by resuscitation of animals may depend on the presence of adrenal hormones. At the same time it was impossible to exclude the effect of operation trauma, involving both injury to the nerve plexuses in the region of the operative field and failure of the function of the adrenals. For this reason in the next series of experiments the animals were taken 4 days after adrenalectomy. In the postoperative period, small doses of cortisone were injected and a high salt diet was given. The experiments showed that in these animals a relatively rapid restoration of the cardiac activity and of respiration were observed during resuscitation after clinical death (Fig. 2). However the normalization of the vital functions later on was also inadequate here.

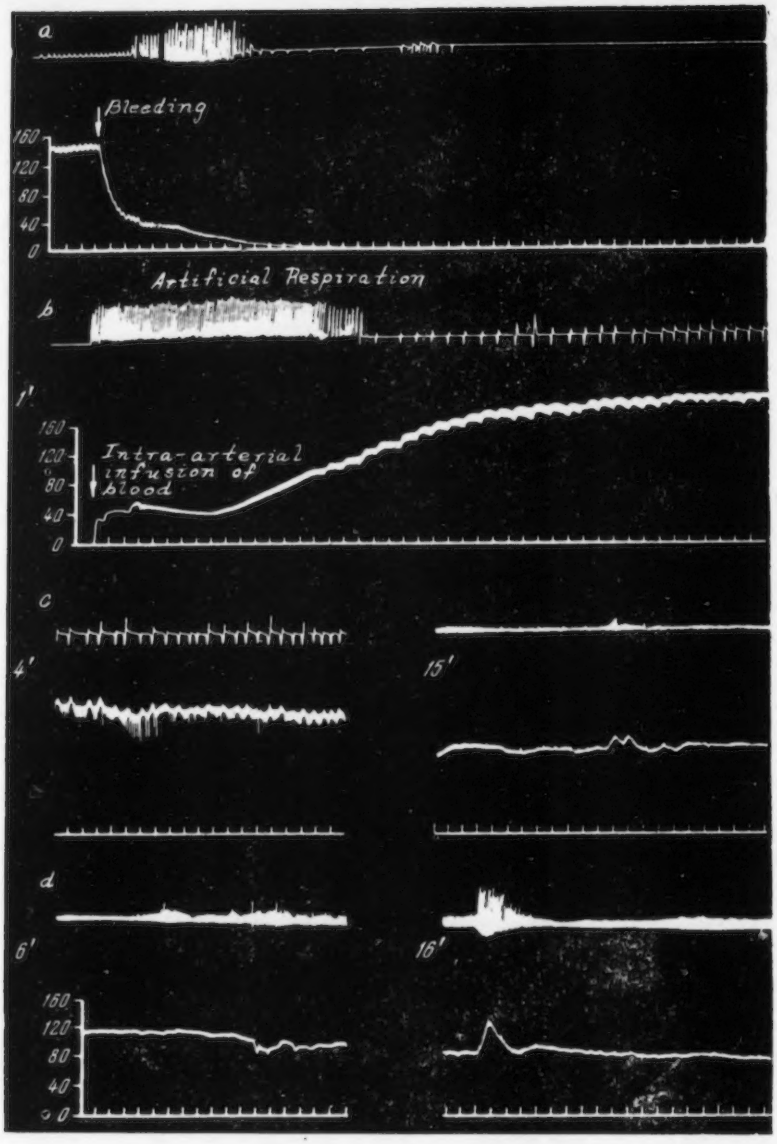


Fig. 3. Restoration of the vital functions after clinical death of a cat in an experiment dated May 24, 1958, in which large doses of cortisone were injected regularly in the postoperative period. Legend as in Fig. 1.

In the last series of experiments (c), after adrenalectomy the animals received a large quantity of cortisone throughout the entire postoperative period. During resuscitation after fatal blood-loss in these animals, the level of the blood pressure and the respiration were rapidly restored (Fig. 3). The normalization of the functions in the recovery period in this series of experiments was carried further. It is interesting to compare the results with those of resuscitation of normal healthy animals, as obtained in a joint investigation by G. L. Lyuban and one of us. In Table 1 are shown the mean values of the main indices characterizing the stage of dying and the first period of restoration of the vital functions after clinical death.

It must be pointed out that the adrenalectomized cats died after a smaller blood-loss than the healthy cats.

TABLE 1

Indices Characterizing the Stage of Dying and Resuscitation of Cats after Clinical Death

Series of experiments	Duration			Number of experiments	
	of stage of dying	of clinical death	of restoration of respiration	total	in which adrenalin was used
Healthy animals	7′42″	5′	6′30″	10	2
Series A	6′40″	4′38″	3′50″	10	3
» B	7′36″	4′48″	7′58″	10	4
» C	8′40″	4′45″	4′17″	10	3

TABLE 2

Changes in the Arterial Pressure of Cats During the First Hour after Resuscitation

Series of experiments	Arterial pressure in mm of mercury (mean values)								
	original	after resuscitation							
		at the moment of appearance of spontaneous respiration	5′	10′	15′	20′	30′	40′	60′
Healthy animals	155	100	165	140	125	120	130	137	125
Series A	98	84	124	100	96	85	85	62	40
» B	104	66	93	95	87	84	81	86	56
» C	114	68	105	128	121	104	109	106	88

TABLE 3

Length of Survival of Adrenalectomized Cats Enduring Clinical Death

Experiment No.	Length of survival of animals, series of experiments		
	A	B	C
1	1 hr 07 min	1 hr 05 min	14 hr
2	0 hr 57 min	2 hr 30 min	10 hr
3	1 hr 07 min	4 hr	7 hr
4	2 hr 40 min	3 hr 40 min	15 hr
5	1 hr 50 min	1 hr 10 min	13 hr
6	1 hr 50 min	1 hr 05 min	10 hr
7	2 hr 07 min	2 hr 40 min	14 hr
8	1 hr 28 min	2 hr	3 hr
9	1 hr 10 min	8 hr	5 hr 45 min
10	1 hr 25 min	1 hr 50 min	3 hr
Average	1 hr 35 min	2 hr 50 min	9 hr 30 min

In view of the equal duration of the stage of dying and of clinical death in the adrenalectomized animals, the difference which was found in the restoration of respiration must evidently be attributed to variations in the management of the postoperative period. This stage of affairs calls for further experimental investigation.

As may be seen from Table 2, the blood pressure in the adrenalectomized animals at the beginning of the experiment was lower than in the healthy cats, but was close to the physiological normal level.

In the period of restoration of the vital functions after clinical death in the series A experiments (16-17 hours after adrenalectomy) and the series B experiments (4 days after, with small doses of cortisone) the arterial pressure fell considerably, and reached at the 60th minute a level which threatened the vital activity of the animal.

Attention was drawn to the slight increase in the blood pressure of healthy animals 30-40 minutes after the appearance of spontaneous respiration. At this time the ocular reflexes had been restored in the animals, the respiration quickened, the muscle tone increased, and a motor reaction was periodically observed, which showed the restoration of the functions of the midbrain [9, 10].

In adrenalectomized animals at the 30th-40th minute of resuscitation, an increase of blood pressure, although very slight, was observed in the experiments in which large doses of cortisone were given (series C).

Later on all the adrenalectomized animals which had endured clinical death died at various times after the moment of reestablishment of spontaneous respiration (Table 3).

As the experiments showed, the adrenalectomized cats could be brought out of a state of clinical death by means of V. A. Negovskii's method. In the first period of resuscitation, judging by the restoration of the arterial pressure and the respiration, there were no special signs of adrenal deficiency. This possibly was due to the profound inhibition of the central nervous system. Characteristically the animal showed at this period considerably increased resistance to various pathological agents [5, 6, 7, 12 and others]. Subsequently, as the functions of the midbrain and diencephalon were restored, signs of deficinecy of adrenal hormones began to appear

In the experiments in which the adrenalectomized animals were "saturated" with cortisone, normalization of the functions during resuscitation was more successful. A relationship was thus revealed between the restoration of the vital functions after clinical death and the presence of the adrenal cortical hormone in the body.

In this connection it is relevant to mention the views of A. A. Bogomolets in support of the role of the adrenals in the pathogenesis of traumatic shock [1]: "It is highly probably that depression of the internal secretory activity of the adrenal cortex arises secondarily in the animal, as the result of severe disturbances of the function of the nervous system, but the inevitability of repercussion on the nervous system of the disturbances of the internal secretory activity of the adrenal cortex is perfectly obvious."

The use of cortisone in a severe terminal state, such as the period of restoration of the vital functions after clinical death when increased demands are made on the neurohumoral regulation and the absolute or relative adrenal deficiency may have an unfavorable influence on the body, appears to us to be promising. As we know, hormone therapy has justified itself in clinical practice, especially in the prophylaxis and treatment of postoperative shock [13, 14].

SUMMARY

The restoration of vital functions (Professor V. A. Negovskii's method) was studied following lethal desanguination in adrenalectomized cats.

Several groups of animals were experimented upon: observation of the first group of animals was conducted 16-17 hours after adrenalectomy, adrenalectomy with administration of saline solution and cortisone (30 mg/kg of body weight per course), on the third – 4 days after adrenalectomy with administration of saline solution and large doses of cortisone (70 mg/kg of body weight per course).

The restoration of vital functions was best in adrenalectomized animals which received large doses of cortisone. The authors believe that cortisone is of value in the therapy of terminal conditions.

LITERATURE CITED

[1] A. A. Bogomolets, Collected Works, 1, 13-164, Kiev, 1956.*

[2] M. Gaevskaya-Sokolova, The Carbohydrate Metabolism of the Cerebral Cortex During Extinction and Restoration of the Vital Functions of the Body. Author's abstract of Dissertation for Doctorate, Moscow, 1955.

* In Russian.

[3] A. G. Ginetsinskii and A. V. Lebedinskii, A Course in Normal Physiology, Moscow, 1956.*

[4] P. D. Gorizontov, Patol. Fiziol. i Éksptl. Terap. 1, 4, 3-10 (1957).

[5] M. G. Kolpakov, First Plenum of the Siberian Society of Pathophysiologists, 66-67, Tomsk, 1957.*

[6] Yu. M. Levin, First Plenum of the Siberian Society of Pathophysiologists, 71-72, Tomsk, 1957.*

[7] G. L. Lyuban, Transactions of a Conference on the Pathophysiology and Therapy of Terminal States, p. 166, Moscow, 1954;* Byull. Éksptl. Biol. i Med. No. 7, 19-22 (1956).**

[8] N. B. Medvedeva, Experimental Endocrinology, Kiev, 1946.*

[9] V. A. Negovskii, The Pathophysiology and Therapy of Agony and Clinical Death, Moscow, 1954.*

[10] I. R. Petrov, Oxygen Starvation of the Brain, Leningrad, 1949.*

[11] V. S. Shapot, Uspekhi Sovremennoi Biol. 34, 2, 244-267 (1952).

[12] G. S. Yakobson, First Plenum of the Siberian Society of Pathophysiologists, 69-70, Tomsk, 1957.*

[13] A. Gerhards, Chirug. 28, 7 (1957).

[14] F. Lagrot and G. Antonie, Mem. Acad. Chir. 83, 17-18 (1957).

[15] H. J. Selye, Clin. Endocrinol. 6, 117-230 (1946).

*In Russian.

**Original Russian pagination. See C. B. Translation.

THE EFFECT OF PITUITRIN ON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS

A. A. Belous

From the Division of Pharmacology (Head - Active Member AMN SSSR S. V. Anichkov) of the Institute of Experimental Medicine (Director - Corresponding Member AMN SSSR D. A. Biryukov), Leningrad

(Received May 19, 1958. Presented by Active Member AMN SSSR S. V. Anichkov)

When studying the effect of intravenous injection of pituitrin in dogs and monkeys [2], we noticed that besides the development of a persistent hypertension by these animals, changes in the activity of the heart were observed, of the coronary insufficiency type. These disturbances of hemodynamics after administration of pituitrin suggested to us the possible effect of an extract of the posterior lobe of the pituitary on the development of atherosclerosis and on cholesterol metabolism.

Experiments were carried out on rabbits in order to discover the effect of pituitrin on the course of experimental atherosclerosis. The experimental atherosclerosis was induced in these animals by the method of Academician N. N. Anichkov [1] (oral administration of cholesterol in oily solution).

EXPERIMENTAL METHOD

For the experiment 25 rabbits of the same species (chinchilla) were used, divided into 3 groups. All the animals were kept on an ordinary diet.

The first group of animals (7 rabbits) received cholesterol only by mouth in a dose of 0.5 g in oily solution daily.

The second group of animals (12 rabbits) received the same dose of cholesterol daily and, in addition, 0.5-0.6 ml of pituitrin P was injected intravenously.

The third series of animals (6 rabbits) received every day pituitrin alone by intravenous injection of a dose of 0.5-0.6 ml.

Blood was taken from all the experimental animals before administration of cholesterol concentration was determined. Before the beginning of the experiment 3 separate serum cholesterol estimations were carried out on each rabbit at intervals of one week. In addition the cholesterol concentration was determined in 2 control rabbits which received neither preparation. These rabbits acted as controls for the third group of animals which received pituitrin alone.

Throughout the whole experimental period, the serum cholesterol level was systematically determined every 2-3 weeks in all the rabbits simultaneously.

According to data in the literature, a severe disturbance of cholesterol metabolism and development of atherosclerosis were observed after feeding rabbits for 4 months on cholesterol. In our experiments, as a rule, rabbits which survived for 4 months were killed for examination of the lungs, liver, kidneys and adrenals. The aorta was next isolated throughout the extent of its thoracic and abdominal portions and removed together with the heart. The aorta was incised along its whole length, treated histologically and stained with Sudan III.

TABLE 1

Blood Cholesterol Concentration in Rabbits Receiving Cholesterol Alone (in mg%)

Rabbit No.	Normal	Experimental time from starting cholesterol feeding (in months)				Death of animals
		1	2	3	4	
1	130	550	950	1175	1000	Killed after 4 months
2	130	1000	1250	1400	1250	The same
4	100	800	750	970	1250	" "
6	80	800	800	1050	980	" "
7	120	800	1200	1800	1400	" "
8	105	780	1100	—	—	Killed after 2 months 8 days
Mean		770	1000	1280	1180	

TABLE 2

Blood Cholesterol Concentration in Rabbits Receiving Cholesterol and Pituitrin Concurrently (in mg%)

Rabbit No.	Normal	Time from beginning of experiment (in months)				Death of animals
		1	2	3	4	
3	130	310	830	—	—	Died after 1 month 21 days
5	70	200	400	490	—	Died after 3 months 4 days
9	190	710	1100	1100	1000	Killed after 4 months
11	115	1100	650	500	500	» » 4 »
12	100	900	1350	—	—	Died after 2 months 7 days
13	120	800	960	—	—	» » 2 » 25 »
23	80	760	1000	—	—	» » 2 » 10 »
24	105	880	880	—	—	» » 2 » 10 »
25	50	680	330	—	—	» » 2 » 10 »
Mean		590	830	—	—	

All rabbits dying or killed before this period were also examined post mortem and the aorta was examined macroscopically.

EXPERIMENTAL RESULTS

The results of our investigations were as follows: From the determination of the blood cholesterol concentration in the group of animals receiving cholesterol alone, we observed a considerable increase in the cholesterol in the first month from the beginning of cholesterol feeding. With continuing cholesterol feeding, the blood cholesterol reached its maximum level at the end of the third month. The monthly values for some of the rabbits are shown in Table 1.

In the group of rabbits receiving cholesterol and pituitrin concurrently we observed a more variegated picture. The results of these investigations are shown in Table 2. As may be seen from Table 2, in rabbits Nos. 3, 5 and 25 a small increase in the blood cholesterol was observed in the first month from the start of administration of pituitrin and cholesterol. In rabbit No. 11, on the other hand, a considerable increase in the cholesterol was observed in the first month (to 1100 mg %), but this began to fall in the second month, and at

the end of the third month it had fallen to 500 mg %. A fall in the cholesterol level in the second month also took place in rabbit No. 25. In rabbit No. 5 cholesteremia developed slowly, and at the end of the third month it had reached 490 mg %.

In the third group of rabbits, receiving pituitrin alone daily for 4 months, we observed no increase in the blood cholesterol: the normal values were not exceeded. In rabbit No. 19 only did we find a threefold rise in the cholesterol content, i.e. to 300 mg %.

In the experiments in which both pituitrin and cholesterol were given (second group of animals) the mortality among the animals was high. Of the 12 rabbits included in the experiment only 2 survived for the test period of 4 months. These were killed at the end of the experiment. The remaining rabbits died at various times.

Anatomical examinations showed that the death of the rabbits was the result of an inflammatory process in the lungs, and also that in the rabbits receiving both pituitrin and cholesterol the inflammation of the lungs was suppurative in character, with necrotic destruction of the lung tissues. In the group of animals receiving cholesterol alone, only one rabbit (No. 20) died, and the picture of inflammation of the lungs differed sharply from that observed in the rabbits of the second group receiving pituitrin and cholesterol concurrently: no foci of suppuration were found In the third group of rabbits, receiving pituitrin alone, only one rabbit (No. 19) died. In this rabbit the inflammation of the lungs resembled the picture of lobar pneumonia: hepatization of the right lung was present.

Macroscopic examination of the aortas of the rabbits killed after feeding for 4 months on cholesterol alone showed that in all the animals the walls of the aorta were grossly thickened, and almost the entire internal surface as far as halfway along the abdominal portion of the aorta, was covered with a continuous layer of atherosclerotic plaques. Particularly large plaques were observed at the orifice and in the thoracic portion of the aorta. In the abdominal aorta, large collections of cholesterol plaques were disseminated, and in its lower portion only isolated plaques were found.

The results of the macroscopic examination of the aortas from rabbits receiving cholesterol and pituitrin concurrently showed a somewhat different pattern of formation of cholesterol plaques. In rabbits Nos. 9 and 11, killed after 4 months, the atherosclerotic changes in the aorta were less pronounced than in the rabbits receiving cholesterol alone and killed at the same times.

In these rabbits (Nos. 9 and 11) the aorta was covered with a continuous layer of cholesterol plaques only in the upper third of its thoracic part, and more distally – to half way along the abdominal aorta – individual groups of plaques were scattered, whereas in the lower third of the abdominal aorta either no cholesterol plaques were observed or occasional isolated ones. Under these circumstances it must be pointed out that the wall of the thoracic aorta, covered with a continuous layer of plaques, was less severely thickened than in rabbits receiving cholesterol alone.

In rabbits dying at various times after receiving both cholesterol and pituitrin, the following macroscopic picture was observed: the aorta of rabbit No. 5, dying after 3 months 4 days, was covered with individual groups of plaques only in the upper third of its thoracic part, and the wall of the aorta was not thickened. In rabbit No. 13, dying after 2 months 25 days, only at its orifice was the aorta covered with a continuous layer of cholesterol plaques, in the thoracic and upper third of the abdominal parts of the aorta there were isolated groups of cholesterol plaques, and in the remaining portion of the aorta no plaques were to be found. In rabbits dying after 2 months 7 days (No. 12) and 1 month 21 days (No. 3), no cholesterol plaques could be found in the aorta. In rabbits killed after 2 months 10 days (Nos. 23 and 24), the walls of the aorta from the orifice to halfway along the abdominal portion were covered with a continuous, thin, translucent layer of cholesterol plaques, but in the lower half of the abdominal aorta no plaques were found. The wall of the aorta was not thickened anywhere in its extent. In rabbit No. 25, killed at the same period (2 months 10 days), cholesterol plaques were observed in isolated groups as far as one third of the way along the abdominal aorta.

In the third group of animals, receiving pituitrin alone, on macroscopic examination of the aorta no cholesterol plaques were observed.

From a comparison of our results it can be seen that not only does pituitrin not enhance experimental cholesteremia and experimental cholesterol atherosclerosis but, on the contrary, it tends to inhibit these processes.

SUMMARY

Experimental atherosclerosis was induced in rabbits by Academician N. N. Anichikov's methods. It was established that pituitrin not only does not intensify the experimental cholesteremia, but on the contrary, reduces it in comparison with its value in control experiments.

LITERATURE CITED

[1] N. N. Anichikov, Transactions of the Second All-Union Congress of Pathologists, pp. 315-319, Baku, [In Russian], 1932.

[2] A. A. Belous and G. O. Magakyan, Byull. Éksptl. Biol. i Med. Supplement to No. 1, 17-21 (1957).*

*Original Russian pagination. See C. B. Translation.

EXPERIMENTAL INDUCTION OF ATHEROSCLEROSIS IN RABBITS

N. A. Yushchenko

From the Laboratory of Experimental Therapy (Head - Dr. Med. Sci. A. M. Chernukh) of the Institute of Normal and Pathological Physiology (Director - Active Member AMN SSSR V. N. Chernigovskii) of the AMN SSSR, Moscow

(Received August 23, 1957. Presented by Active Member AMN SSSR N. N. Anichkov)

In the study of the pathogenesis of atherosclerosis and in its treatment the experimental production of this disease in laboratory animals is very important. For this purpose the method most widely used until the present time has been that suggested by N. N. Anichkov [1]. The production of experimental atherosclerosis in rabbits by N. N. Anichkov's original method takes 3 months or longer [2-6].

Having in mind research on the subject of coronary atherosclerosis, we have attempted to induce well-marked experimental atherosclerosis in rabbits in a shorter time than was previously possible, by varying the dosage of cholesterol.

EXPERIMENTAL METHOD

Usually the cholesterol is administered to the rabbits through a tube, with a large volume of vegetable oil, which has the additional effect of altering the usual dietary regime; introduction of the tube may also cause some degree of trauma to the animals, especially in the hands of persons who have not mastered the technique. We therefore gave cholesterol in the form of a mixture with grated carrot. The animals ate this mixture readily, and always ate it completely.

The rabbits received daily 3 g of cholesterol in 100 g of grated carrot, which made up one third of the daily ration of 300 g of food. Our data on the daily dose of cholesterol of 1% of the weight of the ration agreed with those of Bragdon [8].

For the experiment we selected 12 male rabbits of the chinchilla breed, weighing from 1.9 to 3.0 kg. We divided them into two groups: the rabbits of the first group received cholesterol in the proportion described daily for 30-35 days; the rabbits of the second group – for 60-70 days (see Table).

EXPERIMENTAL RESULTS

The animals of the 1st group showed no abnormal tendencies in their behavior: they ate all their food, and in the course of the experiment they gained in weight significantly more than did the control animals. The rabbits of the 2nd group showed some degree of sluggishness, which appeared on about the 40th day from the beginning of feeding, but they ate their food completely and did not lose weight in the course of the experiment (see Table).

Pathological anatomical examination of the 4 animals of the 1st group that were killed revealed thickly disseminated individual large plaques, yellowish white in color, on the entire internal surface of the aorta, which in places had the appearance of large confluent areas. In 2 rabbits of this group a small number of yellow and white plaques were foundon the internal surface of the aorta throughout its extent. In all 6 rabbits of the 1st group the liver was slightly enlarged; its edge was rounded off with the surface, and on section it had the color of clay.

Results of the Experiments to Produce Atherosclerosis in Rabbits

Rabbit No.	Weight of rabbit (in g)		Number of days of feeding	Macroscopic changes in the aorta
	at the start of the experiment	at the end of the experiment		
43	3000	3200	31	++
477	2000	2200	35	+++
34	1950	2050	34	++
52	2000	2200	34	+++
39	2100	2350	32	+
54	2050	2500	35	+++
89	2900	2850	67	+++
453	2600	2900	65	+++
224	2700	2850	70	+++
430	2700	2900	60	+++
455	2800	2650	70	+++
495	1900	2250	60	++

Conventional designation of the atherosclerotic changes in the aorta: +++ severe changes, ++ moderate, + slight.

General appearance of the aorta of a rabbit with experimentally induced atherosclerosis.
a) Lipoid stains and atherosclerotic plaques in the aorta of rabbit No. 34 after 34 days of feeding with cholesterol; b) the same in the aorta of rabbit No. 430 after 60 days of feeding with cholesterol.

At postmortem examination of the rabbits of the 2nd group, in all 6 animals on the internal surface of the thoracic and abdominal portions of the aorta and also on the branches emerging from it were observed thickly disseminated, small linear and larger elongated plaques, yellowish or white in color, some of which were firm in consistency.

In 5 of the 6 rabbits we also observed whitish or yellowish plaques on the aortic semilunar valves and on the mitral valves of the heart. In all 6 rabbits the liver was somewhat enlarged and its edge was rounded; superficially and on section it was clay colored.

Characteristic pathologicoanatomical changes of experimental atherosclerosis in the rabbit were thus obtained in all the animals; they were particularly expressed in the animals of the 2nd group.

The experimental results described demonstrate that the method adopted is perfectly suitable for the production of experimental atherosclerosis in rabbits in shorter periods of time.

This communication is a part of a paper which we have written describing a study of the mechanisms of coronary insufficiency in a chronic experiment.

SUMMARY

By changing the usual dose of cholesterol it was possible to shorten the period of experimental induction of atherosclerosis from 3-6 months to 2 months. The daily dose of cholesterol equalled 1% of the total weight of food fed to the rabbits. This was continued

for 2 months. Taking into consideration that large amounts of vegetable oil change the usual diet of the animals, cholesterol was mixed with grated carrots (3 gm of cholesterol to 100 gm of grated carrots).

LITERATURE CITED

[1] N. N. Anichkov, in the book: Regional Pathological Anatomy, by A. I. Abrikosov, II, p. 380, Moscow, 1940.

[2] N. N. Anichkov, Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie, begründet von E. Ziegler, 1913, Bd. 56, S. 379.

[3] M. V. Bavina, The Effect of Vitamin D_2 on the Lipids of the Blood and the Development of Experimental Atherosclerosis, Dissertation, 1952. *

[4] K. G. Volkova, Atherosclerosis (Conference on the Problem of Atherosclerosis, Leningrad, October 30 to November 1, 1952), p. 68, Moscow, 1953.*

[5] Yu. T. Pushkar', The Effect of Choline and of Certain Drugs (Luminal and Phenamine) on the Development of Experimental Atherosclerosis, Dissertation, 1953.*

[6] T. A. Sinitsina, Atherosclerosis (Conference on the Problem of Atherosclerosis, Leningrad, October 30 to November 1, 1952), p. 68, Moscow, 1953.*

[7] V. S. Smolenskii, The Effect of Hypertension on the Development of Experimental Atherosclerosis, Dissertation, 1952.*

[8] Y. H. Bragdon, Circulation, 1952, vol. 5, 646.

*In Russian.

CHANGES IN THE AMMONIA AND GLUTAMIC ACID CONTENTS OF THE BRAIN OF ANIMALS FOLLOWING SURGICAL INTERFERENCE WITH LIVER FUNCTION

N. V. Veselkin and B. G. Gordon

From the Laboratory of Biochemistry of the Central Nervous System (Head – Corresponding Member AMN SSSR G. E. Vladimirov), I. P. Pavlov Institute of Physiology, AN SSSR, Leningrad

(Received June 6, 1958. Presented by Active Member AN SSSR V. N. Chernigovskii)

The effects on central nervous activity of disturbances in liver function were demonstrated many years ago by the researches of I. P. Pavlov, I. Zalesskii and M. Nencki [10], whose findings have since been confirmed by numerous workers, including ourselves, in both clinical and experimental investigations.

It is known that liver diseases are associated with various metabolic disturbances. Very serious disturbances of ammonia metabolism are encountered in such conditions as hepatic cirrhosis, hepatic coma, and establishment of an Eck fistula. At the same time, disturbances in the activity of the central nervous system are observed, being particularly pronounced in hepatic coma.

Many workers support the view that development of the comatose condition is due chiefly to the toxic action of ammonia on the nervous system [14, 15, 19]. For this reason, the use of glutamic acid for the treatment of hepatic coma has been introduced of recent years, and we have found it useful for the prevention of poisoning by ammonia, in animal experiments [1] and for the treatment of patients suffering from liver diseases.

I. P. Pavlov and M. Nencki [10] and S. S. Salazkin [11] showed that the output of urinary ammonia of dogs with an Eck-Pavlov fistula rose after feeding with meat or ammonium citrate. It was also found that the glutamine content of the brain of dogs rose after hepatectomy [17]. A rise in the ammonia content of the brain has also been observed in prolonged narcolepsy, due to impairment of the urea-synthesizing function of the liver [9]. Determinations of differences between the ammonia contents of arterial and venous blood of patients suffering from liver disease have established that ammonia is taken up by the brain when the ammonia content of arterial blood exceeds 0.1 mg % [14].

The object of the present research was to find out whether the preformed ammonia content of the brain rises following impairment of liver function. In view of its important role in ammonia metabolism, we also determined the glutamine content of the brain.

EXPERIMENTAL METHODS

Surgical procedures were applied for infliction of injury to the liver, such as are used clinically for the treatment of liver diseases. In an earlier reseach we had damaged the liver by carbon tetrachloride poisoning.*

In order to reproduce the conditions arising after an Eck-Pavlov operation we applied the operation of constriction of the portal vein, followed by its ligation, as devised by M. V. Shepelev for cats [12]. This operation

* "Effects of disturbances of hepatic function on nitrogen metabolism," Trudy Inst. Fiziol. im. I. P. Pavlova, 1958, Vol. VIII.

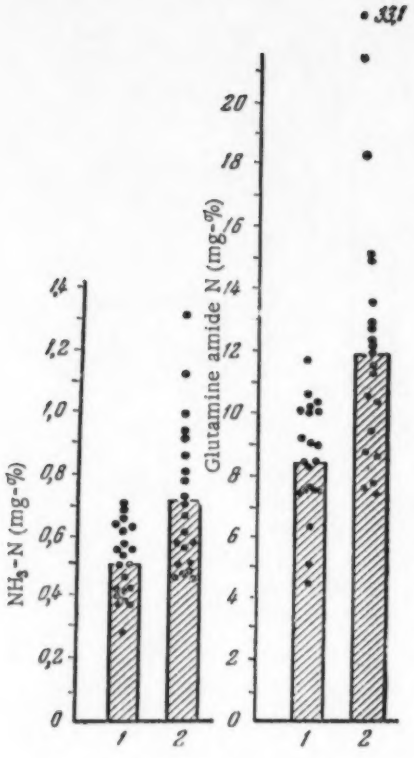


Fig. 1. Ammonia and glutamine contents of the cerebral cortex of cats before and after ligature of the portal vein.
1) Normal values; 2) after ligature of the portal vein. The value of 33.1 mg% of glutamine was not taken into account in calculating the mean value.

causes a rise in blood ammonia content [7]. In the given experiments, the portal vein was ligated 3 weeks after its preliminary constriction, and the brain of the cat was removed 6-8 days later, for ammonia and glutamine determination. The cat was rolled up firmly in a towel, and, after it had ceased to struggle, the head was plunged deeply into liquid oxygen. After it had been frozen we severed the head, extracted the frozen brain, and analyzed it for ammonia and glutamine. The analytical procedure used was that of E. A. Vladimirova [5].

EXPERIMENTAL RESULTS

The results of our experiments are shown in Figure 1. It is evident that ligature of the portal vein of cats causes an increase in the ammonia and glutamine contents of the brain (by 42 and 40%, respectively, on the average). The analytical values found in different experiments showed a very wide scatter, due probably to differences in the rate and the extent of establishment of collateral circulation after the preliminary stenosing operation. Comparison of the analytical results with those of inspection of the abdominal cavitiy during the second operation and after decapitation showed that the ammonia and glutamine contents of the brain rose when the collateral circulation was achieved chiefly by development of portocaval anastomoses, but not when it involved chiefly hepatopetal anastomoses.

In our next series of experiments we attempted to perform the operations on rats. Application of the constricting ligature to the portal vein of rats presented no difficulties, but we found that access to the portal vein for its total ligation 2-3 weeks later was very difficult, owing to formation of extensive adhesions of the omentum and the viscera to each other and to the liver. We were therefore obliged, in some operations, to restrict ourselves to a one-stage operation: the constriction of the portal vein was made as complete as possible, short of total obliteration (with the risk of causing the death of the animal, which followed in some cases), with the expectation that the gradual obliteration of the vein would follow, as is indicated by the literature [12, 20].

As in the experiments with cats, the values found for the ammonia and glutamine contents of rat brains showed a wide scatter, depending on the nature and extent of the changes in hepatic circulation. In those cases in which the autopsy showed that the portal vein was patent the ammonia and glutamine contents were within the normal range. Where the portal vein was obstructed, and where there was evidence of development of portocaval anastomoses, we found a certain increase in the ammonia and glutamine contents of the brain (Fig. 2, 3).

In a special series of experiments the rats were subjected to partial (two thirds) hepatectomy. This operation is easily performed, and is practically bloodless. The brain was removed for analysis on the fifth day after the operation, since by that time the effect of operational trauma are no longer in evidence, while only slight regeneration of hepatic tissue will have taken place [18]. Since this procedure did not give sufficiently conclusive results (see Fig. 2, 4) we increased the extent of interference with liver function by applying a constricting ligature to the portal vein, during the partial hepatectomy operation. We then found an appreciable rise in both the ammonia and the glutamine content of the brain (see Fig. 2,5).

In the next series of experiments the liver was totally excluded from the circulation, by removing the stomach and intestines, and ligating all the vessels entering or leaving the liver. The survival time after this operation was usually 5-6 hours, or somewhat more. The brain was taken for analysis 3-5 hours after the operation, and the results showed a definite elevation of the ammonia and glutamine contents (see Fig. 2, 6).

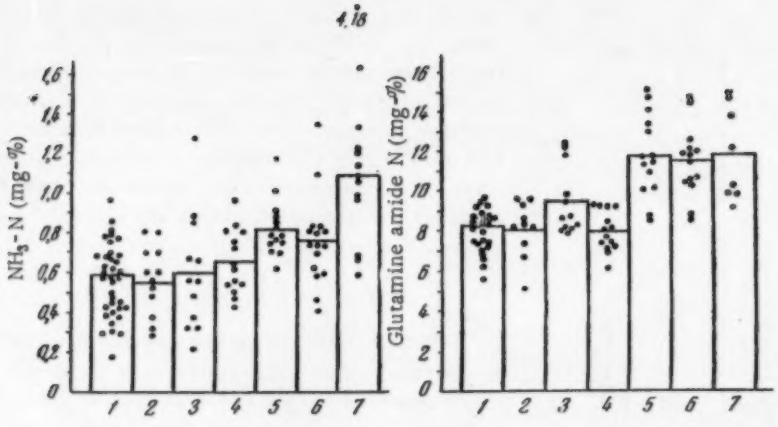


Fig. 2. Ammonia and glutamine contents of the cerebral cortex of normal and operated rates.
1) Normal; 2) control laparotomy; 3) constriction of the portal vein; 4) partial hepatectomy; 5) partial hepatectomy with constriction of the portal vein; 6) by-passing the circulation of the liver; 7) subtotal (90%) hepatectomy. Mean ammonia content – 4.18.

In the last series we performed a subtotal hepatectomy, removing 90% of the initial weight of the liver, and these experiments showed that the ammonia and glutamine contents of the brain had risen within 4-5 hours of the operation (see Fig. 2, 7).

It is well known, that apart from the ammonia entering the brain with its blood supply, ammonia may be rapidly formed in the brain, and its content may rise, following various forms of stimulation of the central nervous system; for this reason special precautions have to be taken to avoid exciting the rats when the normal brain ammonia content is to be determined [6], since otherwise a wide scatter of the results is obtained, with a high mean value for the normal ammonia content (see Fig. 2, 1).

It appears from our results that, however great might be the effects of differences in the handling and behavior of animals on the ammonia and glutamine contents of their brains, the values found for animals with liver damage were all much higher than for normal animals, even in the chronic experiments (see Figs. 1 and 2, 5). This difference may quite certainly be ascribed to the raised content of ammonia in the blood entering the brain. In the acute experiments, although the animals rapidly regained consciousness (within 5-10 minutes), and assumed their usual sitting posture, or even performed active movements, they then became progressively less active, their legs splayed out and became unable to support the weight of the body, and shortly before their death the animals lay motionless on their sides. Thus in our acute experiments the rats were in a state of depression for several hours, but regardless of this the ammonia content of the brain was elevated, due to the ammonia entering the brain with its blood supply (see Fig. 2, 6 and 7).

We observed much the same effect in our earlier experiments on dogs with an Eck-Pavlov fistula; meat feeding caused a depressive state in these animals, and was associated with a high blood-ammonia level [2]. As far back as 1894, N. O. Yurinskii [13], working under the direction of I. P. Pavlov, investigated the reasons for the stimulatory and inhibitory effects of annonia on the central nervous system, and, on the basis of his own findings and of those of other authors, he concluded that it had a dual activity. É. É. Martinson and L. Ya. Tyakhepyl'd [9], in their experiments on prolonged narcotic sleep (7-15 days), found considerably raised levels of ammonia in the brain, which could, in their opinion, cause both depression and stimulation. Incidentally, we think it significant that these authors found that animals in barbiture-induced sleep were highly resisistant to the toxic effects of large doses of ammonium chloride, as compared with control animals. We found a similar effect in dogs with an Eck-Pavlov fistula; when meat was fed after prolonged previous administration of sodium bromide the characteristic features of meat intoxication did not develop – there was no vomiting, and the dogs slept most of the time, while the blood ammonia level was raised.

What is the mechanism of the toxic action of ammonia on the brain?

Apart from the view that this is due to interference with synthesis and breakdown of acetylocholine, there is a different approach to the elucidation of the mechanism whereby ammonia exerts its toxic effect. Various authors have ascribed the neurologic changes in the central nervous system to interference with the normal functioning of oxidative processes in the brain. According to these authors, ammonia present in the blood entering the brain enters into combination not only with glutamic acid, forming glutamine, but also with α-oxoglutaric acid, which is an important substrate of the tricarboxylic acid cycle. By combining with large amounts of α-oxoglutaric acid (to form glutamic acid, and, from this, glutamine), ammonia causes a lowering of the amounts of the subsequent stages of the cycle, and hence to a lowered oxygen uptake, and this interferes with the oxidative metabolism of the brain [8, 14, 15, 19]. When glutamine is formed exclusively from preformed glutamic acid these authors consider that the oxygen uptake is raised, and that the free glutamic acid reserves of the brain are gradually used up. This view is supported by the findings of S. Bessman and A. Bessman [14] and of I. Fazekas, H. Ticktin, W. Ehrmantraut et al. [16] that the oxygen uptake of the brain in hepatic insufficiency and hepatic coma is in the fact lowered, and of E. Flock [17] that there is a large increase in the glutamine content of the brain of hepatectomized dogs, while the glutamic acid content remains unchanged.

Our results also show a considerable increase in brain glutamine content due either to carbon tetrachloride poisoning or to surgical intervention, such as ligature of the portal vein (see Fig. 1), partial hepatectomy coupled with constriction of the portal vein, by-passing the liver from the circulation, and subtotal hepatectomy (90% extirpation) (see Fig. 2, 5-7).

The distrubances in central nervous activity observed in patients suffering from hepatic insufficiency may thus be ascribable, to a certain extent, to the toxic effect of the raised content of ammonia in the brain. For this reason the treatment of such condition should be such as to promote the elimination of the excess ammonia from the organism.

SUMMARY

The ammonia and glutamine contents of the brain of cats and rats were found to be raised following surgically induced hepatic insufficiency (by constriction and ligature of the portal vein, by exclusion of the liver from the circulation, and by partial and subtotal hepatectomy). The effects of ammonia on the central nervous system are related not only to its combination with glutamic acid, but also to its reaction with α-oxoglutarate; removal of this tricarboxylic acid cycle metabolite may interfere with the normal oxidative metabolism of brain tissue.

LITERATURE CITED

[1] N. V. Veselkin and B. G. Gordon, Doklady Akad. Nauk SSSR 107, No. 2, 333-336 (1956).

[2] N. V. Veselkin and B. G. Gordon, Trudy Inst. Fiziol. im. I. P. Pavlova, 5, 425-439 (1956).

[3] N. V. Veselkin and B. G. Gordon, Byull. Éksper. Biol. i Med. 39, No. 5, 43-45 (1955).

[4] N. V. Veselkin and B. G. Gordon, Voprosy Med. Khim. 1, No. 6, 437-443 (1955).

[5] E. A. Vladimirova, Byull. Éksper. Biol. i Med. 29, No. 3, 219-221 (1950).

[6] E. A. Vladimirova, Doklady Akad. Nauk SSSR 106, No. 5, 937-940 (1956).*

[7] B. G. Gordon and M. V. Shepelev, Byull. Éksper. Biol. i Med. 42, No. 11, 23-28 (1956).*

[8] M. F. Gulyi, L. A. Mikhailovskaya and R. G. Degtyar', Proc. 8th All-Union Congress of Physical., Biochem. and Pharmacol. pp. 194-195 (Moscow, 1955);** Proc. 1st Sci. Meeting on Problems of Fats in Nutrition, p. 29 (Leningrad, 1958).

[9] É. É. Martinson and L. Ya. Tyakhepyl'd, Problems of the Biochemistry of the Nervous System, pp. 176-188 (Kiev, 1957).**

[10] I. P. Pavlov (with I. Zalesskii and M. Nencki) Complete Collected Works, Vol. 2, Book 1, 287 (Moscow-Leningrad, 1951).**

*Original Russian pagination. See C. B. Translation.

**In Russian.

[11] S. S. Salazkin, "Role of the liver in biosynthesis of urea in mammals" (Thesis, St. Petersburg, 1897). •

[12] M. V. Shepelev, "Collateral circulation in the portal vein system of the cat" (Thesis, Leningrad, 1952). •

[13] N. O. Yurinskii, "Pharmacology of ammonium chloride" (Thesis, St. Petersburg, 1894). •

[14] S. P. Bessman and A. N. Bessman, J. of Clin. Invest. v. 34, 622 (1955).

[15] S. P. Bessman, I. F. Fazekas and A. N. Bessman, Proc. Soc. Exper. Biol. a. Med., v. 85, 66 (1954).

[16] I. F. Fazekas, H. E. Ticktin and W. R. Ehrmantraut et al., Am. J. Med., v. 21, 843 (1956).

[17] E. V. Flock et al., J. Biol. Chem. v. 200, 529 (1953).

[18] R. D. Harkness, J. Physiol. v. 117, 287 (1952).

[19] W. V. McDermott, R. D. Adams and A. G. Riddel, Ann. Surg. v. 140, 539 (1954).

[20] H. L. Popper and N. C. Jefferson, Proc. Soc. Exper. Biol. a. Med., v. 85, 67 (1954).

• In Russian.

MITOGENETIC ANALYSIS OF THE PROTEIN SUBSTRATE OF PROTOPLASM

PART II. DIVIDING CELLS AND CELLS WHICH HAVE EMERGED FROM THE "MERISTEMATOUS" STATE

N. S. Slavina

From the Mitogenesis Laboratory (Head – A. A. Gurvich), Institute of Normal and Pathological Physiology (Director – Active Member AMN SSSR V. N. Chernigovskii), AMN SSSR, Moscow

(Received August 15, 1957. Presented by Active Member AMN SSSR V. N. Chernigovskii)

The methods at present available for the study of molecular changes proceeding in the protoplasm do not permit us to follow the course of rapidly succeeding changes in its chemical composition, and, in particular, of changes in the relative locations of molecules and their complexes. Such methods can inform us only of certain transiently existing states of the protoplasm – "structures".

TABLE 1

Spectra of the Phenolic Groups of Tyrosine, $R-C_6H_4OH$, and of the Carbonyl Groups $R-C=O$, from the Radiation of 18-hour and 3-day Yeast Cultures

Functional group	Wave length, A	Mean effect, %		Number of experiments (1) / (2)
		18-hour culture (1)	3-day culture (2)	
C = O (I)	1960—1975	58,2	69,2	5/5
C = O (II)	2100—2110	59	64,2	5/5
C_6H_4OH	2770—2790	70,5	62,8	21/19
Adjacent to C=O	1955—1960	2,2	61,4	5/5
	1975—1980	—6,6	76,4	5/5
	2090—2100	2,1	0.2*	5/5
	2110—2120	4,8	59,4	5/5
Adjacent to C_6H_4OH	2760—2770	—1	69,6	9/10
	2790—2880	2,7	112,2	10/10

*The band did not broaden towards the short wave side.

There is, however, experimental evidence that, in many of the basic processes taking place in the protoplasm, great importance is attached to weak, readily broken bonds between peptide chains. The number of such bonds, and their significance, appear to vary widely, depending on the functional state of the system, and may in many cases lead to establishment of conditions approaching localized reversible gelation of the protoplasm.

The fact that within the short period of mitotic division all the cell elements undergo reconstruction leads naturally to the concept that the microscopically observable processes of breakdown and reformation of microstructures have their analogous submicroscopic molecular counterparts. It was natural to postulate that an important part is played in mitosis by processes leading to disaggregation, i.e. to achievement of maximum mobility of protein and nucleoprotein elements of the cytoplasm and nucleus. One would, on the contrary, anticipate a lower mobility of the substrate, i.e. higher degree of association of molecular complexes, in the cells of tissues in which cell division is not proceeding.

TABLE 2

R−C=O and R−C_6H_4OH Bands in Newt Fin Radiation Spectra

Functional group	Wave length, A	Average effect, %		Number of experiments	
		larvae (I)	adult newts (II)	on larvae	on adult newts
CO (I)	1960—1975	65	40	9	5
CO (II)	2100—2110	59	49	7	5
C_6H_4OH (I)	2720—2730	57	70	7	7
C_6H_4OH (II)	2770—2780	47	65	7	8
C_6H_4OH (III)	2780—2790	60	60	8	8
Adjacent to CO(I)	1955—1960	1	43	6	5
The same	1975—1980	2	47	6	5
Adjacent to CO (II)	2090—2100	2,5	46	6	5
Adjacent to $C_6H_4OH_{(I)}$	2710—2720	0,3	61	7	7
The same	2730—2740	3	60	7	7
Adjacent to $C_6H_4OH_{(II)}$	2760—2770	0,6	57	7	8
Adjacent to $C_6H_4OH_{(III)}$	2790—2800	2	50	8	8

Our experiments were performed on material in two physiological states − the state of intensive multiplication of cells, and the state corresponding to physiological emergence from the "meristematous" state.

The mitogenetic method permits of the investigation of the extent of disaggregation and breakdown of large protein complexes held together by weak intermolecular bonds (bond energy 4-8 kcal / mole), in experiments performed on living, undamaged organisms.

Conclusions as to the presence and the extent of intermolecular bonding were based on analysis of mitogenetic spectra of selective scattering. Substrates not possessing intermolecular quasichemical bonds give spectra with narrow bands, corresponding closely with those of the standards; the bands become broader when quasichemical bond formation takes place in the substrate (see Part I).

As for the data obtained from our investigation of gelatin, we chose, for our in vivo investigations, one of the functional groups of the peptide chain, viz. the phenolic group of tyrosine and the carbonyl group of the peptide chain itself. As found for the standard prepared in our laboratory, the functional group R−C=O is represented in the selective scattering spectrum by bands at 1960-1975 and 2100 -2110 A. The spectrum of the phenolic group of tyrosine may be considered, with sufficient accuracy for the purposes of this research, to include bands at 2720-2730 and 2770-2790 A.

1. Experiments on Cultures of the Yeast Saccharomycetes Cerevisiae

We set up two identical cultures of the organisms in beer wort. One of the cultures was taken for examination after 18 hours, at the stage of intensive multiplication of the cells, and the other culture was examined after 3-4 days. By that time, not only had cell division ceased, but the fermentation process was also practically completed, so that the cells were all located at the bottom of the vessel, as a dense sediment. Both cultures were maintained at 27-28°.

The cells were washed with tap water before being taken for spectral analysis in the form of slightly opalescent aqueous suspensions, using water of the same temperature.

TABLE 3

R−C=O Bands of Radiation of the Labial Mucous Membrane of Guinea Pigs

Functional group	Wave length, A	Effect, %	
		in newborn guinea pigs	in adult guinea pigs
CO (I)	1960—1975	39, 43, 52	31, 54, 45
CO (II)	2100—2110	39, 50, 48	55, 50, 41
Adjacent to CO (I)	1955—1960	2, −2, 3	36, 54, 48
The same	1975—1980	0, 2, 3	39, 35, 43
Adjacent to CO (II)	2090—2100	2, 1, −2	43, 40, 54

We examined only the 2770-2790 A band due to the phenolic group of tyrosine, since the 2720-2730 A band is subject to background interference in 18-hour cultures. As is evident from the data of Table 1, the characteristic spectra of the functional groups R−C=O and R−C_6H_4OH are represented by narrower bands in 18-hour than in 3-day cultures. The conclusion may hence be drawn, on the basis of the data found for model systems (Part I), that the process of ageing of the cultures − their physiological emergence from the stage of multiplication − coincides with the formation of weak, quasichemical bonds which interlink peptide chains, to form two- and three-dimensional structures.

2. Experiments on Newts

We examined the outer epithelium of the tail fin, which is a uniform stratified formation not containing glandular elements. Both larval and adult forms were used for the experiments.

In the larval stage, the tail fin epithelium emits so-called spontaneous mitogenetic radiation. The experiments on adult newts were conducted during the autumn and winter, on the same individuals which had undergone metamorphosis in the laboratory. The same functional groups were taken for spectral examination, viz. the R−C=O carbonyl groups, and the R−C_6H_4 phenolic group.

Apart from the analysis of selective scattering spectra, we examined the spontaneous radiation spectra of larval tissue. A certain lengthening of exposure was required for the detection of spontaneous radiation bands.

The newt fin was screened with a sheet of thick paper having a small aperture (0.3 × 0.3 cm) centered in front of the collimator slit of the spectrograph. The intensity of the ultraviolet radiation (from a physical source) incident on the tissue was lowered by interposing a number of mat quartz filters between the tissue and the radiation source. This is an essential condition of the application of the method of selective spectrum analysis to the study of aggregates present in living tissues, such as protein substrates in the crystalline or gel forms, since the general background of scattered radiation unavoidable in such experiments greatly weakens the specific effects, making them almost subthreshold even for the sensitive biological method of detection of mitogenetic radiation.

An active region is to be found in the emission spectrum of the fin of newt larvae, contiguous with the longer wave length part of one of the characteristic carbonyl group bands (2100-2110 A). In comparing this region of the spectrum for both larval and adult newts we could only make use of the short wave end of the band. There is no overlapping of either of the bands due to the phenolic group of tyrosine, so that both of them are available for comparative studies.

It is evident from the data of Table 2 that the selective scattering spectrum of the fin of adult newts is distinguished by the breadth of its bands, as compared with those of larval tissue. In other words, much of the same results were obtained with two such disparate materials as are yeast cultures and newt fins − the peptide chains of the cells of actively growing tissues are relatively little interlinked; in contrast, in tissues in which the cells have, even if only temporarily, ceased to multiply the peptide substrates are more highly aggregated, due to formation of weak intermolecular bonds.

3. Experiments on Guinea Pigs

We examined the surface epithelium of the mucous membrane of the lips of guinea pigs, which spontaneously

TABLE 4

R−C=O Bands of Radiation of the Corneal Epithelium of Rabbits

Functional group	Wave length, A	Effect, %
$CO_{(I)}$	1960—1975	43, 27, 58, 64
$CO_{(II)}$	2100—2110	45, 78, 80, 62
Adjacent to $CO_{(I)}$	1955—1960	17, 15, 10, 10
The same	1975—1980	7, 4, −9, −3
Adjacent to $CO_{(II)}$	2090—2100	0, 7, 3, 2
The same	2110—2120	−1, −5, 2, 3

emits mitogenic radiation. For spectrum analysis we everted the lower lip, covered it with a celloidin screen, and immobolized it. An aperture in the screen, exposing a small area of the lip surface (0.3 × 0.3 cm) was centered against the collimator slit of the spectrograph.

In this series of experiments we examined the carbonyl group bands in the spontaneous radiation spectrum of newborn guinea pigs (ages from 1 to 3 days) and of adult guinea pigs (weighing from 500 to 1000 g). The width of the first carbonyl group band of the radiation from the lips of newborn guinea pigs was very close to that of the corresponding bands found for actively dividing yeast cultures and for larval newt tail fins; the second band overlapped an active region of the spectrum (2110-2120 A), toward the side of increasing wave lengths. For this reason we confined our analysis of the second band to the short wave region of the spectrum only, without entering into a consideration of its origin.

We found that both bands were broadened in the emission spectrum of the mucous membrane of lips of adult guinea pigs (Table 3). In other words, cessation of division of mammalian epithelial cells is associated with the same effect as was seen in the other cases − the peptide substrate undergoes aggregation, i.e. weak bonds are formed between peptide chains.

4. Experiments on Rabbits

In this series of experiments we studied the spontaneous emission spectrum of rabbit cornea, which is known to be "meristem" with a high mitotic index, in which cell division persists in the adult animal. Animals of medium size and weight were taken for spectrum analysis, which was confined to the carbonyl group.

As can be seen from Table 4, both of the R−C=O bands are narrow, i.e. they resemble those found in the previous experiments on biological material in the "meristematous" state. This finding shows that constant physiological "meristem" is associated in the adult organism with absence of intermolecular bonds between peptide chains, as was found in other "meristems" of different kinds.

The application of mitogenetic spectrum analysis has thus enabled us to establish that in all the organisms examined the "meristematous" state is associated with presence of narrow bands due to the functional groups R−C:O and $R-C_6H_4OH$, while in a state of maturity these bands become more diffuse.

Comparison with the corresponding bands of the radiation spectrum of gelatin gel and sol shows that narrowness of the bands may be interpreted as indicating mutual independence of the peptide chains (in sols), whereas their broadening is associated with formation of weak (quasichemical) intermolecular bonds (in gels). It is thus very probable that a characteristic feature of cells during mitosis (and probably also interkinesis) is the smaller degree of aggregation of the peptide and protein substrates, i.e. there is much less interlinking of peptide chains. On the other hand, tissues which have emerged from the "meristematous" state are characterized by formation of considerable numbers of weak intermolecular bonds, leading to the formation of two- and even three-dimensional peptide and protein chain structures.

It is evident that mitosis is a process involving considerable and co-ordinated reconstruction of all the elements of the cell substrate, and should be associated with a preponderance of free, i.e. mobile, peptide chains.

SUMMARY

It has been shown, by means of mitogenetic spectrum analysis that during the proliferative stage of cells (yeast, newt larvae, newborn guinea pigs), and in proliferating tissues of adult animals (guinea pig labial epithelium, rabbit cornea) there is relatively little interlinking of cytoplasmic peptide chains. This is shown by the relative narrowness of the spectral bands due to the functional groups $R-C=O$ and $R-C_6H_4OH$. Where active proliferation has ceased, the peptide chains are interlinked by weak molecular bonds, giving rise to two- and three-dimensional structures.

The mitotic process thus involves profound and co-ordinated reconstruction of all cell components.

LITERATURE CITED

[1] A. G. Gurvich and L. D. Gurvich, Mitogenetic Radiation, Its Physicochemical Bases, and Its Applications to Biology and Medicine (Moscow, 1945).*

[2] A. G. Gurvich and L. D. Gurvich, Introduction to the Study of Mitogenesis (Moscow, 1948).*

* In Russian.

EFFECT OF IONIZING RADIATION ON THE URIC ACID AND ALLANTOIN CONTENTS OF THE BLOOD AND URINE OF ANIMALS

T. A. Fedorova, V. P. Fedotov and N. A. Mkrtumova (Moscow)

(Received May 15, 1958. Presented by Active Member AMN SSSR A. E. Braunshtein)

The object of the present reseach was to investigate the effects of lethal doses of γ- and α-radiation on the contents in the blood and urine of different species of animals of the end products of nucleic acid metabolism – uric acid and allantoin.

EXPERIMENTAL METHODS

Our experiments were performed on 12 dogs and 8 rats.

Male dogs weighing from 24 to 30 kg were maintained on a strictly regulated diet. Blood uric acid was determined by the method of Caraway [3], and blood allantoin by that of Young, MacPherson and Conway [5, 6].

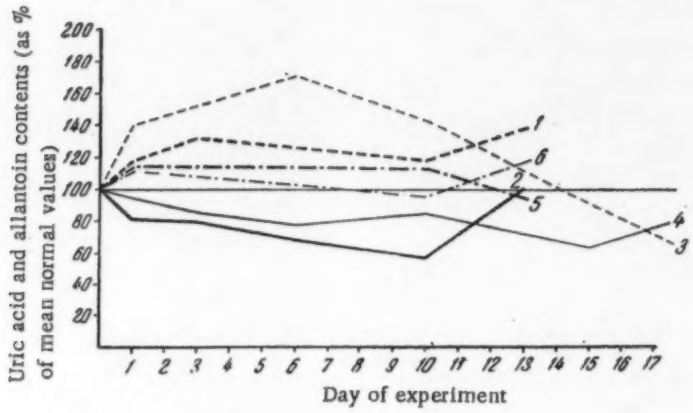


Fig. 1. Blood uric acid and allantoin contents of dogs after exposure to γ-ray dosages of 350 and 400 r.
Allantoin (1) and uric acid (2) after exposure to 400 r of radiation; allantoin (3) and uric acid (4) after exposure to 350 r of radiation; allantoin (5) and uric acid (6) in control animals.

The analysis were performed 5-6 times, at 2-5 day intervals, for each dog before it was exposed to irradiation.

The first group consisted of 4 dogs, which were subjected to total γ-ray irradiation in single doese of 350 r (2 dogs) and 400 r (2 dogs). Blood samples (7 ml) were taken for analysis from the femoral vein and artery. The

TABLE 1

Uric Acid and Allantoin Contents of Blood from the Portal and Hepatic Veins, and from the Arterial System of Normal Dogs

Name of dog	Uric acid						Allantoin					
	in arterial blood	in portal vein blood	secreted from the intestinal wall (+)	in the blood flowing to the liver	in hepatic vein blood	removed by the liver (−)	in arterial blood	in portal vein blood	secreted from the intestinal wall (+)	in the blood flowing to the liver	in hepatic vein blood	removed by the liver (−)
	mg-%	mg-%	%	mg-%	mg-%	%	mg-%	mg-%	%	mg-%	mg-%	%
Dozor	0,57	0,68	+19,3	0,64	0,51	−20,3	3,35	3,58	+ 6,9	3,49	3,11	−10,9
Volchok	0,51	0,75	+47,0	0,67	0,54	−19,3	3,33	3,50	+ 5,1	3,44	2,90	−15,4
Migai	0,44	0,56	+27,3	0,52	0,48	− 7,7	2,68	2,98	+11,2	2,86	2,65	− 7,4
Mean . . .	0,51	0,66	+29,4	0,61	0,51	−16,4	3,12	3,35	+7,7	3,26	2,89	−11,5

TABLE 2

Uric Acid and Allantoin Contents of Blood from the Portal and Hepatic Veins and from the Arterial System of a Control Dog and of Dogs Following Irradiation

Condition of animal	Uric acid						Allantoin					
	in arterial blood	in portal vein blood	secreted by the intestinal wall (+)	in the blood flowing to the liver	in hepatic vein blood	removed by the liver (−)	in arterial blood	in portal vein blood	secreted by the intestinal wall (+)	in the blood flowing to the liver	in hepatic vein blood	removed by the liver (−)
	mg-%	mg-%	%	mg-%	mg-%	%	mg-%	mg-%	%	mg-%	mg-%	%
Normal values for the control dogs	0,44	0,56	+27,3	0,52	0,48	− 7,7	2,68	2,98	+11,2	2,88	2,65	− 7,4
1st day of experiment	0,43	0,57	+32,6	0,52	0,47	− 9,6	3,32	3,78	+13,9	3,67	3,14	−13,5
2nd " " "	0,43	0,53	+23,3	0,50	0,48	− 4,0	2,86	2,96	+ 3,5	2,93	2,80	− 4,4
3rd " " "	—	—	—	—	—	—	2,36	2,52	+ 6,8	2,47	2,24	− 9,3
7th " " "	0,39	0,58	+48,7	0,52	0,55	+ 5,8	2,80	3,42	+22,4	3,21	3,14	− 2,2
10th " " "	0,51	0,66	+29,4	0,61	0,51	−16,4	3,12	3,35	+ 7,7	3,26	2,89	−11,5
Normal value (mean) for 3 dogs												
After irradiation:												
1st day	0,55	0,76	+38,2	0,69	0,42	−39,1	3,02	3,72	+23,2	3,49	3,23	− 7,5
3rd "	0,50	0,60	+20,0	0,57	0,32	−43,9	3,08	3,50	+13,6	3,36	3,12	− 7,1
7th "	0,39	0,58	+48,7	0,52	0,32	−38,5	3.29	3,73	+13,4	3,58	3,45	− 3,6
10th "	0,38	0,55	+44,7	0,49	0,30	−38,8	3,47	3,64	+4,9	3,58	3,14	−12,0

second group consisted of 3 dogs, on which an angiostomy had been performed by E. S. London's procedure [1], with application of skin cannulae (by I. A. Pigalev's procedure [2]) to the portal and hepatic veins; these dogs were exposed to a single γ-ray irradiation of the whole body, at a dosage of 400 r. Blood samples were taken from the portal and hepatic veins and from the femoral artery. Since in unit time 2/3 of the blood supply of the liver enters via the portal vein, and 1/3 via the hepatic artery (E. S. London), the overall average concentration of uric acid and allantoin in the blood entering the liver can hence be calculated.

Irradiation was performed using an ÉGO-2 equipment, delivering a dose of 506-538 r/min., with an exposure time of 40.6-47.4 seconds. The animals died on the 14-17th day after irradiation.

The third group consisted of two dogs, which were treated with polonium (doses of 0.06 mC/kg, administered subcutaneously). The animals died on the 28th and 30th day after the injections.

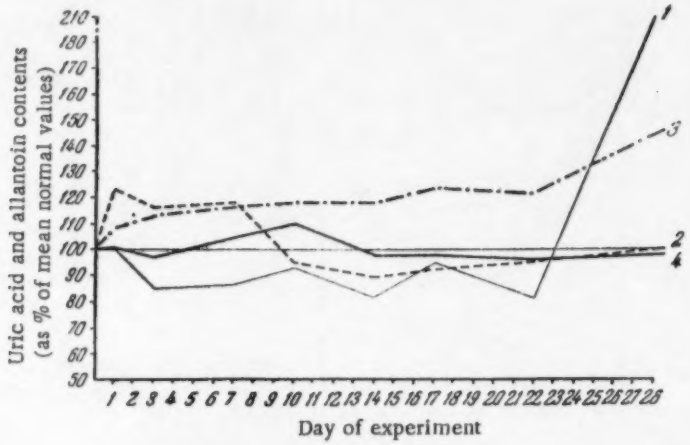


Fig. 2. Mean uric acid and allantoin contents of the blood of two dogs after administration of polonium (at dosage of 0.06 mC/kg), and of a control dog.
Uric acid in polonium-treated dogs (1) and in the control dog (2); allantoin in polonium-treated dogs (3) and in the control dog (4).

Blood samples were taken for analysis at 2-5-day intervals during the course of radiation sickness.

Parallel with each group of experimental animals we analyzed blood samples from a control dog, which was maintained on the same daily food ration as was consumed by one of the irradiated dogs.

Male rats, weighing 180-200 g, were kept in metabolism cages on a standard diet consisting of pellets containing 60% of starch, 20% of casein, 10% of animal fat, 3% of yeast and 5% of salt mixture. Uric acid was determined in the 24-hour urine output, by Borsook's method [4], and allantoin by Young and Conway's method [5]. We analyzed 6-8 24-hour portions of urine from each rat before exposing the animals to irradiation. Four of the eight rats were then given subcutaneous injections of polonium (dosage level 0.1 mC/kg). The animals survived for from 5 to 12 days. The remaining 4 rats served as control animals. They were given the same daily ration as was consumed by the injected rats. The urine of the polonium-treated rats was analyzed daily, beginning with the first day after injection.

EXPERIMENTAL RESULTS

The uric acid contents of arterial and venous blood taken from the dogs of the first group were practically identical before irradiation. The values for arterial blood were 0.54 ± 0.041 to 0.75 ± 0.041 mg %•, giving a

• In the statistical treatment of the numerical data the standard deviation m was derived from the formula $m = \pm \frac{\sigma}{\sqrt{N}}$, where σ is the mean square error, and N is the number of determinations.

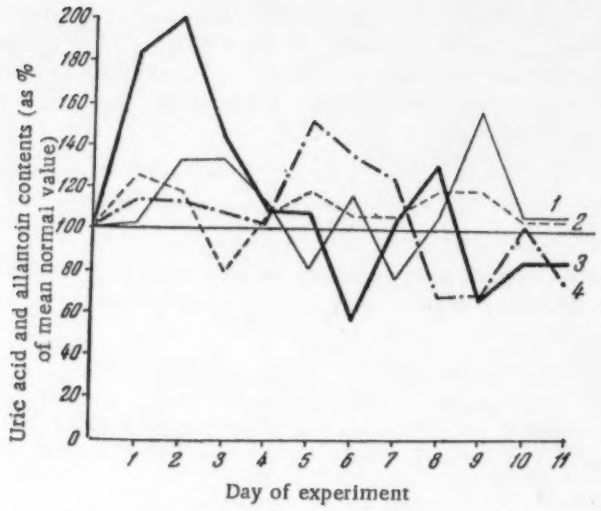


Fig. 3. Mean uric acid and allantoin contents of 24-hour portions of urine from four rats, after administration of polonium (at dosage of 0.1 mC/ kg), and from four control rats (as percentages of the mean normal value).
Allantoin from polonium-treated rats (1) and from the control rats (2); uric acid from polonium-treated rats (3) and from control rats (4).

mean value of 0.62 mg-%. The allantoin content was practically identical for venous and arterial blood, and varied for arterial blood within the range 2.65 ± 0.09 to 3.23 ± 0.088 mg %, giving a mean value of 2.8 mg %.

It may be seen from Fig. 1 that the blood uric acid content varied within the normal range for the control animals, with a tendency toward higher values, while the allantoin content rose by 12-14%. The uric acid content of the serum of irradiated dogs fell by 20-30%, on the average, and serum allantoin rose by 25-40%.

The results given by the second group of dogs related to uric acid and allantoin metabolism in the liver and intestinal wall.

It is evident from the data of Table 1 that under normal conditions the highest contents of uric acid and allantoin were found in the blood from the portal vein, as compared with that of the hepatic vein and of the arterial system.

It may be supposed that the processes of synthesis of uric acid and formation of allantoin take place chiefly in the walls of the small intestine. That these substances are not of exogenous origin is shown by the results of special experiments in which the dogs were fasted for 4 days. Under such conditions, which completely excluded the possibility that uric acid and allantoin could have been derived from ingested food, both of these substances continued to be secreted into the blood stream from the intestine.

In normal animals the liver removes uric acid from the blood passing through it (from 6 to 20%), as well as allantoin (from 7 to 16%).

Table 2 shows the corresponding mean results obtained from the same dogs after irradiation and from the control dog. The uric acid content of blood taken from the specified vessels of the control angiostomized dog receiving a restricted food ration during the experiment did not vary beyond the limits of the normal range for this dog. It was not until the 10th day, when the dog showed clear signs of undernourishment, that the uric acid content of hepatic vein blood rose slightly, and that its retention by the liver ceased to be evident. The allantoin content of all the blood samples was high on the 1st and the 10th day of the experiment, and its retention by the liver was smaller on the 3rd and the 10th day.

Apart from the first few days after irradiation with γ-rays, there was a regular decline in the uric acid content of the blood from the various vessels, to a minimum on the 7-10th day. In comparison with the initial mean normal values, the uric acid content of blood from the portal vein fell by 16.7%, of arterial blood by 25.5%, and of hepatic vein blood by 41.2%. The allantoin content was in general raised (except in the case of arterial blood on the 1st and the 3rd days after irradiation).

The amount of uric acid removed from the blood by the liver was 2½ times greater than in normal animals. The amount of allantoin removed by the liver had fallen to a third of the normal amount on the 7th day after irradiation.

The increase in the amount of uric acid removed by the liver, considered in conjunction with the rise in allantoin in hepatic vein blood, indicates that oxidation of uric acid to allantoin in the liver is intensified in irradiated animals.

Production of uric acid in the wall of the small intestine rose slightly as the course of radiation sickness progressed (roughly as for the control dog, on the 10th day), as is shown by the rise in uric acid content of portal vein blood. Formation of allantoin in the walls of the small intestine of irradiated dogs also rose as the disease progressed (except on the 10th day), whereas in the control dog there was no increase in allantoin from this source until the 10th day.

Fig. 2 shows the mean value found for two dogs treated with polonium (third group), with the corresponding results for the control dog. The uric acid content of the arterial blood of these dogs varied, before the injections, within the range 0.54 ± 0.063 to 0.61 ± 0.012, mean value 0.64 mg %; the allantoin content varied from 2.65 ± 0.023 to 3.21 ± 0.144 mg %, mean value 2.95 mg %. The blood uric acid of these dogs fell by 15% from the 3rd day after injection of polonium, and remained at a low level until the death of the animals. The allantoin content rose from the second day after administration of polonium, being 29% above the normal on the 17th day, and 40% above normal at death. The uric acid content found for the undernourished control dog rose, on the contrary, from the first days of the experiment, and did not revert to the normal value until the 9th day, after which it was somewhat below normal. There was only a slight rise in allantoin content, which in general varied within normal limits.

Fig. 3 illustrates the results found for urinary excretion of uric acid and allantoin at various times after injection of polonium into a group of 4 rats, and for the undernourished control rats. The uric acid content of a 24-hour portion of urine varied normally within the range 0.89-2.67 mg, mean value 1.75 mg; the allantoin content varied within the range 22.6-37.5 mg, mean value 30 mg.

Urinary output of uric acid and of allantoin rose from the 1st to the 3rd day, inclusive, after administration of polonium. During the subsequent 5 days the values fell to normal, or somewhat below. Uric acid excretion fell, and allantoin excretion rose, in the period immediately preceding the death of the animals.

It is of interest that on the 5-6th day after administration of polonium, when uric acid excretion had fallen by 45%, that of the control rats had risen by 50%. This difference is indicative of intensification of the process of oxidation of uric acid at the height of radiation sickness.

In general, the range of uric acid values found in plutonium treated rats was wider than for the control group.

Our results give clear evidence of the effects of radiation, both external and internal, on the contents of the chief end products of purine metabolism in the blood and urine of different species of mammals, and permit the differentiation of these effects from those due to diminished food intake associated with radiation sickness.

SUMMARY

The uric acid and allantoin contents of the blood and urine of dogs and rats receiving lethal doses of γ- and α-radiation have been determined. The uric acid contents of the blood and urine fell by 15-45% during the course of radiation sickness, while the allantoin contents rose by 30-50%. The food rations of the control animals were restricted to those of the irradiated ones, and were quantitatively inadequate; the uric acid content of the blood and urine rose under these conditions by 17-20%, and the allantoin content by 12-15%.

Experiments on rats with a circulatory by-pass of the liver showed that at the height of radiation sickness the liver removed more uric acid from the blood than normally, but put out more allantoin.

LITERATURE CITED

[1] E. S. London, Angiostomie and Organstoffwechsel (Moscow, 1935).

[2] I. A. Pigalev, Problems of Pathology and Metabolism, 13-22 (Leningrad, 1950).*

[3] W. T. Caraway, Am. J. Clin. Path. v. 25, 840-845 (1955).

[4] H. Borsook, J. Biol. Chem. v. 110, 481 (1935).

[5] E. G. Young and C. F. Conway, J. Biol. chem. v. 142, 839-835 (1942).

[6] E. G. Young, M. G. Macpherson and C. F. Conway, J. Biol. chem. v. 152, 245-253 (1944).

*In Russian.

REDUCTION OF METHEMOGLOBIN BY MEANS OF GLUTAMIC ACID

A. M. Genkin and M. S. Volkov

From the Department of Biochemistry (Head – Prof. S. A. Brailovskii), Sverdlovsk Medical Institute (Director – Prof. A. F. Zverev)

(Received July 15, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

The use of a number of reducing substances has been proposed for the conversion to hemoglobin of methemoglobin, which can arise in the human organism by the action of various industrial poisons (potassium chlorate, nitrites, arsine, etc.) and of drugs (amyl nitrite, sulfanilamides), as well as in toxemias of pregnancy.

TABLE 1

Effect of Sodium Glutamate on the Methemoglobin-Producing Action of Sodium Nitrite

No. of experiment	Time elapsed between injection of sodium nitrite and taking of blood sample	Blood methemoglobin content (%)		% Diminution in methemoglobin content in the experimental group of rats
		control group	experimental group	
1	120	56,8	31,4	44,8
2	85	67,3	45,8	32,0
3	52	67,1	44,4	33,8
4	52	62,7	40,5	35,5
5	65	75,8	47,6	37,2
6	40	80,6	39,3	51,3
7	65	77,4	46,4	40,0
8	33	82,6	49,6	40,0
9	33	74,6	40,9	45,3
10	45	77,0	46,4	39,7
Mean	—	72,2	43,0	40,0

Of the earlier drugs used for the treatment of methemoglobinemia, methylene blue has been the most widely applied, both in experimental work and clinically [2, 6, 9, 13]. Although it rapidly reduces methemoglobin to hemoglobin, methylene blue is not without its drawbacks, since it also gives rise to methemoglobin formation, forming, according to different authors, from 8 to 24% of methemoglobin [6, 10].

Lactic and ascorbic acids are well known to be active reductants of methemoglobin in vitro [1, 12], but these substances were found to be without action in animal experiments [7, 11, 13]. It has recently been shown [3] that glutamic acid greatly raises the resistance of organisms to deficiency of oxygen in the atmosphere. An analogous effect is found in hypoxic conditions, due to administration of sodium nitrite [4]. Furthermore, simultaneous administration of glutamic acid protects rats from the effects of a lethal dose of sodium nitrite.

These results suggested that glutamic acid may affect the process of methemoglobin formation in blood. The present paper is devoted to an investigation of this possibility. We have been unable to find any references in the literature to the action of glutamic acid on blood methemoglobin.

EXPERIMENTAL METHODS

The animal material consisted of adult rats. Methemoglobinemia was induced by subcutaneous injections of sodium nitrite, at a dosage level of 10 mg per 100 g body weight. Our experiments showed that this dose invariably caused the death of the animals. The control rats were decapitated when they displayed preterminal convulsions, and blood methemoglobin was determined. Sodium glutamate (100 mg per 100 g bodyweight) was injected together with sodium nitrite into the experimental rats.

TABLE 2

Effect of Adding Sodium Nitrite and Sodium Glutamate to Blood on Methemoglobin Formation

No. of experiment	methemoglobin content of blood (%) after adding to it	
	sodium nitrite	sodium nitrite + sodium glutamate
1	70,1	19,8
2	74,7	28,1
3	52,4	20,3
4	60,5	25,7
5	66,0	22,7
6	68,8	18,6
7	65,8	20,3
8	67,0	17,2
9	49,3	9,4
10	58,3	16,0
Mean	63,3	19,8

Methemoglobin was determined by a modification of Gorn's method [7]. We used a photoelectric colorimeter with a red filter. We did not use 0.25% ammonia solution for hemolysis and dilution of blood as recommended by Gorn, because according to our findings and to literature references [5] conversion of methemoglobin into hemoglobin is accelerated in alkaline solution (pH > 8), which also transform some of the methemoglobin into alkaline hematin. We effected hemolysis with water alone, and then added sufficient phosphate buffer (pH = 7.4) to bring the concentration of the blood in the solution to 2.66%, as recommended by Gorn.

RESULTS OF EXPERIMENTS

Injection of sodium nitrite into the control animals (10 rats) was followed by uneasiness and then somnolence, the breathing became superficial, and convulsions heralded the onset of death. This took place within 33-120 minutes after injection. The methemoglobin content of blood taken during the agonal period ranged from 56.8 to 82.6%, mean value 72.2%.

The animals of the experimental group given glutamate together with sodium nitrite showed no symptoms, except, possibly slight drowsiness in some of them. Not one of the ten rats went into convulsions, Their blood methemoglobin ranged from 31.4 to 49.6%, mean value 43%, which was 40% lower than in the control rats.

Thus the methemoglobin-producing action of sodium nitrite is very considerably reduced by simultaneous injection of sodium glutamate (Table 1).

We thought it would be of interest to ascertain whether this effect of glutamic acid was exerted only in the

whole animal, or whether it could also be shown in blood withdrawn from animals. For this purpose blood taken from normal rats was hemolyzed and diluted, as before. To 7.5 ml of diluted hemolyzate we added 4 mg of sodium nitrite (control), and to another portion we also added 20 mg of sodium glutamate (experiment). Methemoglobin was determined in both solutions after two hours. The results are presented in Table 2.

It is clearly evident from the data of Table 2 that the methemoglobin-producing effect of sodium nitrite is to a considerable extent inhibited in the presence of sodium glutamate. It may be supposed that this action of glutamic acid, exerted both in the whole animal and in shed blood, should be ascribed to promotion of the reverse process of reduction of methemoglobin, rather than to inhibition of conversion of hemoglobin into methemoglobin by action of nitrite. This question will be the subject of a future research.

SUMMARY

Simultaneous administration of sodium glutamate counteracts the effect of a lethal dose of sodium nitrite given to rats. The methemoglobin content of the blood is 40% lower than in unprotected animals in the terminal stage of nitrite poisoning. Addition of sodium nitrite to blood in vitro gives 63.3% conversion to methemoglobin, as compared with 19.8% when sodium glutamate is added simultaneously.

LITERATURE CITED

[1] G. E. Vladimirov and Z. L. Bolotina, Biokhimiya No. 9, 312 (1944).

[2] R. N. Vol'fovskaya and L. É. Gorn, Farmakol. i Toksikol. 17, No. 1, 36 (1954).

[3] A. M. Genkin and N. A. Udintsev, Byull. Éksper. Biol. i Med. 45, No. 5, 58 (1958).*

[4] S. M. Genkin, Treatment of Poisoning with Amino- and Nitro-derivatives of Benzene (Moscow-Leningrad, 1940).**

[5] B. G. Gordon and É. É. Martinson, Byull. Éksper. Biol. i Med., No. 4-5, 56 (1943).

[6] B. G. Gordon, "Action of certain substances on reduction of methemoglobin to hemoglobin" (Thesis, Sverdlovsk, 1944).**

[7] L. É. Gorn, Farmakol. i Toksikol. 14, No. 4, 37 (1951).

[8] A. I. Kagan and A. I. Cherkes, Vrach. Delo, No. 6, 463 (1948).

[9] V. M. Karasik, Sov. Vrach. No. 14, 1051 (1937).

[10] E. M. Navrotskaya, Farmakol. i Toksikol. 15, No. 3, 53 (1952).

[11] I. F. Seits, Biokhimiya, No. 12, 123 (1947).

[12] N. M. Traperznikova, Farmakol. i Toksikol. 14, No. 4, 34 (1951).

[13] V. S. Shapot, Trudy Inst. Fiziol. LGU, No. 19 (1937).

*Original Russian pagination. See C. B. Translation.

**In Russian.

PHARMACOLOGY

CHANGES IN THE LEVEL OF THE ARTERIAL BLOOD PRESSURE IN DOGS DUE TO COMBINATIONS OF HYPOTENSIVE DRUGS

N. P. Speranskaya

From the Laboratory of Pathophysiology and Pharmacology of the Cardiovascular System (Head - Prof. S. V. Andreev) of the Institute of Pharmacology and Chemotherapy (Director - Active Member AMN SSSR V. V. Zakusov) of the AMN SSSR, Moscow

(Received April 17, 1958. Presented by Active Member AMN SSSR V. V. Zakusov)

In recent years the use of combinations of the hypotensive drugs instead of the individual preparations has become more widespread in the treatment of hypertension. The treatment of hypertension by such combinations enables a better therapeutic response to be obtained by doses smaller than those usually given of the individual components; in addition the side effects caused by the drugs are less marked. In the treatment of severe forms of hypertension in particular, a large series of combinations has been suggested, embracing drugs obtained from the root of Rauwolfia serpentina in conjunction with other drugs which lower the blood pressure. Freis [5], for instance, in all cases of severe hypertension, recommends the use of 3 mixtures containing, in different proportions, reserpine, pentaphyrrholidonium, apresoline and prostigmine. Finnerty [4] states that it is possible to obtain a better response by using small doses, and to reduce side effects by giving a combination of rauwiloid and veriloid. The same sort of effect was obtained by a combination of the preparations from Rauwolfia serpentina with hexamethonium and hydralazine [6] and so on. At the same time the search continues for new combinations of hypotensive drugs which, in small doses, would produce a prolonged fall in the arterial blood pressure without side effects.

The aim of the present investigation was to study the effect of a number of combinations of hypotensive drugs on the blood pressure of dogs in chronic experimental conditions.

EXPERIMENTAL METHOD

Experiments were carried out on 7 dogs (5 males and 2 females) weighing from 8 to 25 kg. In two of them a persistent rise in the arterial pressure was produced by bilateral constriction of the renal arteries (the initial systolic pressure varied between limits of 140-190 mm of mercury and the diastolic between limits of 90-120 mm of mercury). In one old dog there was observed to be an increased arterial pressure presumably due to age (variations in the initial level of the sytolic pressure between 130 and 158 mm of mercury and diastolic between 90 and 120 mm of mercury). In 4 dogs the arterial pressure was within normal limits (systolic pressure from 108 to 150 mm of mercury and diastolic from 68 to 90 mm of mercury).

As hypotensive drugs we used increpan (hypotonium) – a drug with a mainly peripheral action on the vessels, obtained by S. V. Andreev and his co-workers from the pancreas of cattle; gendon - a dutch preparation from the firm of Organon Holland, containing the total alkaloids of rauwolfia (main action on the vascular centers) and dibasol – a Soviet preparation, acting both directly on the smooth muscle of the vessel walls and on the central nervous system (S. V. Anichkov). We used the following combinations of the above-mentioned hypotensive drugs: gendon – increpan, gendon – dibasol and increpan – dibasol. All the drugs were given by mouth with minced meat every day for 10 days.

The blood pressure was measured by Korotkov's auscultatory method of the carotid artery which was brought near the surface in a muff of skin. The lowest arterial pressure before the experiment was taken conventionally to be the initial level.

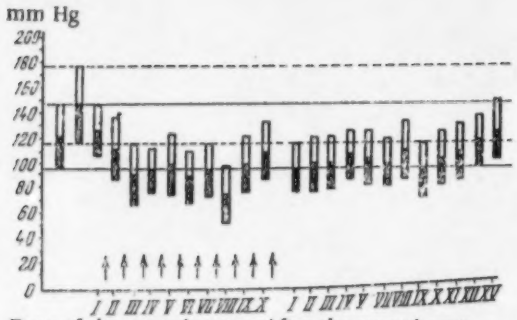


Fig. 1. Changes in the blood pressure in the dog Seryi with experimental renal hypertension in response to the combined oral administration of gendon and increpan for a period of 10 days. Gendon was given in a dose equivalent to 100γ/kg of rauwolfia alkaloids and increpan at the rate of 25 mg/kg daily.
Continuous horizontal lines – the lowest limits of variation of the initial systolic and diastolic pressure; the dotted lines are the highest limits of the same variations. The black-and-white columns show the results of serial measurements of the blood pressure in mm of mercury. Arrow – each single administration of the drugs. The blood pressure began to fall on the 2nd day of administration of the drugs. The maximum fall was observed on the 8th day (the systolic pressure fell by 48 mm and the diastolic by 44 mm of mercury). The blood pressure was restored on the 12th day after conclusion of the course of drugs.

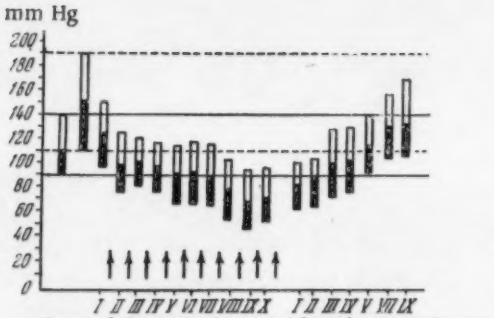


Fig. 2. Changes in the blood pressure of the dog Mishka with experimental renal hypertension under the influence of combined oral administration of gendon and dibasol for 10 days. Gendon was given in a dose of 100γ/kg of rauwiloid alkaloids, and dibasol in a dose of 2.5 mg/kg daily.
Legend as in Fig. 1.

The arterial pressure began to fall on the 2nd day of the course of treatment. The maximum fall was on the 9th day of administration of the drugs (the systolic pressure fell by 44 mm and the diastolic by 40 mm of mercury). The blood pressure was restored on the 5th day after the conclusion of the course of treatment.

The combination gendon – increpan was given to 6 dogs: two with experimental renal hypertension and four with a normal blood pressure. The animals were given daily from $\frac{1}{4}$ to 1 tablet of gendon* (from 20 to 100 γ/kg of the rauwolfia alkaloids) and from 100 to 500 mg of increpan (from 8 to 25 mg/kg). The increpan was given 3 hours after the gendon.

EXPERIMENTAL RESULTS

In all the experiments an obvious fall of the systolic and diastolic pressures was observed, beginning on the 2nd-9th day of taking the drugs and continuing for 7-11 days after the conclusion of the course. The systolic pressure fell by 20-48 mm of mercury (by 18-32%) and the diastolic by 10-44 mm of mercury (by 14-44%) below the initial level depending on the dose of the preparations used in the combination (Fig. 1).

From results obtained previously, the blood pressure remained lowered after the dogs had finished feeding on increpan in those cases only when the drug was given in doses of 25 mg/kg for a longer time (30 days) or when the daily dose was increased to 50-100 mg/kg, i.e. when the dosage was from 2 to 12 times larger than that used in the present investigation.

Administration to a dog (with hypertension due to age) of gendon alone in a dose from 2 to 25 times greater than that used in the combination (2 tablets or 222 γ/kg of alkaloids of rauwolfia daily), although it caused an obvious fall in the systolic (by 28 mm of mercury, or by 22%) and the diastolic pressure (by 22 mm of mercury, or by 24%), this fall was, however, not persistent. The blood pressure was restored on the 2nd day after feeding of this preparation ceased.

Feeding of dogs on a combination of gendon and increpan thus caused a more prolonged hypotensive effect than did the administration of each of the drugs separately, even in large doses.

* Each tablet contains approximately 2 mg of rauwolfia alkaloids.

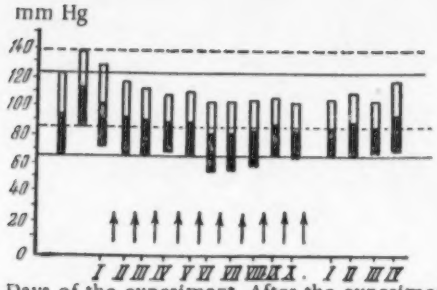


Fig. 3. Changes in the blood pressure of the dog Tsygan (normal blood pressure) under the influence of combined oral administration of increpan and dibasol for 10 days (dose of increpan 18 mg/kg, and of dibasol, 2 mg/kg, daily). Legend as in Fig. 1.

There was an insignificant fall in the systolic pressure. The diastolic pressure remained practically within the limits of the lower level of the initial pressure.

Two dogs (with "age" and renal hypertension) received a combination of gendon and dibasol. Gendon was given in a dose of 1 tablet (100 and 111 γ/kg of alkaloids) and dibasol at the rate of 50 mg (2.5 and 2.8 mg/kg) daily. The interval between the administration of gendon and dibasol was 3 hours.

In both experiments a considerable fall in the arterial pressure was observed, but with rapid restoration to its initial level after cessation of feeding. In the dog Mushka with hypertension due to age, the fall in the diastolic pressure was greater (by 32 mm of mercury, or by 35%) and the fall in the systolic pressure was slight (by 10 mm of mercury, or by 8%). Restoration of the normal value of the systolic pressure started even before the conclusion of the course of the drug – on the 10th day of feeding. The diastolic pressure was restored 3 days after the administration of the drug ceased. In the dog Mishka with renal hypertension, the systolic and diastolic pressures fell by almost the same amount: the systolic by 44 mm of mercury (by 31%), the diastolic by 40 mm of mercury (by 44%). The arterial pressure was restored on the 5th day after the conclusion of the course of feeding with the drug (Fig. 2).

We observed the weakest hypotensive effect after administration of a combination of increpan and dibasol. This combination of drugs was given to 2 dogs with normal arterial pressure. The daily dose of increpan was 18 and 25 mg/kg, and of dibasol 2 and 3 mg/kg. In both experiments an insignificant fall (by 10 mm of mercury) was observed in the diastolic pressure. The systolic pressure was reduced only in the dog Tsygan (by 20 mm of mercury). In one dog the arterial pressure was soon restored, and in the other, on the 2nd day after the conclusion of the course of feeding with the drugs (Fig. 3).

In the Table are summarized the results of oral administration to dogs of various combinations of the hypotensive preparations.

In all the experiments in which gendon was given side effects were observed, shown in the form of drowsiness, weakening of motor activity, to the extent of a considerable degree of adynamia, disturbances of the gait, diarrhea, vomiting and refusal to eat. However, in half the experiments in which the combination of gendon and increpan was used, these manifestations were less severe. These side effects developed on the 4th-10th day of feeding and disappeared on the 4th-10th days after the conclusion of the course, depending on the degree of their severity. The administration of a combination of increpan and dibasol was not accompanied by any side effects.

It can be seen from the results of the experiments that the most prolonged hypotensive action was produced by the combination of the rauwolfia alkaloids with increpan.

In order to achieve a more prolonged fall in the blood pressure, much smaller doses of the drugs forming the combination were required than by the use of each drug separately. A reduction in the severity of the side effects was also observed (in half the experiments).

Oral administration of rauwolfia alkaloids (gendon) and dibasol to dogs was not accompanied by any perceptible increase in the hypotensive action of the rauwolfia alkaloids; nor was the degree of the side effects reduced.

Administration of increpan in combination with dibasol led to a reduction in the hypotensive action of increpan. Thus the simultaneous administration of the rauwolfia alkaloids and increpan evidently showed promise

Changes in the Blood Pressure of Dogs in Chronic Experiments in Which Various Combinations of Hypotensive Drugs Were Administered

Serial No.	Sex	Weight, kg	Type of combination	Sessional dose per kg body weight	Initial blood pressure, mm of mercury		Maximum fall of blood pressure				Duration of fall after cessation of feeding drugs, days
							mm of mercury		%		
					systolic	diastolic	systolic	diastolic	systolic	diastolic	
1	♂	20	Gendon – increpan	100 γ and 25 mg	150	100	48	44	32	44	11
2	♂	20	The same	100 γ and 25 mg	140	90	26	24	19	27	9
3	♀	25	" "	20 γ and 8 mg	110	70	20	20	18	28	9
4	♂	18	" "	28 γ and 11 mg	130	70	36	10	28	14	8
5	♂	8	" "	62 γ and 12 mg	108	68	24	22	22	32	7
6	♂	11	" "	45 γ and 9 mg	128	70	32	20	25	28	7
7	♂	20	Gendon – dibasol	100 γ and 2.5 mg	140	90	44	40	31	44	4
8	♀	18	The same	111 γ and 2.8 mg	130	90	10	32	8	35	3
9	♂	8	Increpan – dibasol	25 and 3 mg	108	68	0	10	0	15	1
10	♂	11	The same	18 and 2 mg	128	70	20	10	16	14	0

in the treatment of hypertension, especially in those cases when treatment by one preparation alone was ineffective. At the same time dibasol weakens the hypotensive action of increpan, a feature which must be remembered when the latter drug is used clinically.

The question of the search for more effective methods of combined administration of the rauwolfia alkaloids and increpan when the blood pressure is raised calls for further experimental study.

SUMMARY

The author studied the effect of the combination of the following hypotensive drugs on the blood pressure of dogs with normal blood pressure and with experimental hypertension: gendon-increpan, gendon-dibasol, increpan-dibasol. The preparations were given per os for 10 days. The blood pressure was measured in the carotid artery enclosed in a skin flap. The best hypotensive effect was obtained from the use of gendon-increpan combination (gendon was given in the dose of 20-100 γ/kg of Rauwolfia serpentina alkaloids, increpan – 8 to 25 mg/kg). Systolic pressure was decreased ty 18-32%, diastolic by 14-44%. The blood pressure was reestablished 7-11 days after the termination of the course of treatment. Each preparation used in combination gave a much lower effect than when used separately. Poor effect was obtained when feeding the dogs by the combination of gendon and dibasol. The use of the increpan-dibasol combination gave a lesser hypotensive effect than administration of increpan alone.

LITERATURE CITED

[1] S. V. Andreev. N. P. Speranskaya, I. D. Kobkova, et.al , Doklady Akad. Nauk SSSR, 105, 4, 848-851 (1955).

[2] S. B. Anichkov (edited by), The Pharmacology of New Drugs, 5-13, Leningrad, 1953.*

[3] N. P. Speranskaya, Proceedings of a Scientific Conference of the Institute of Pharmacology and Chemotherapy, 21-33, Moscow, 1957.*

[4] F. A. Finnerty, Am. J. Med. 17, 629 (1954).

[5] E. D. Freis, Med. Clin. N. Amer. 38, 363 (1954).

[6] M. Markowitz and J. V. Koik, Am. J. Med. Sc. 229, 486 (1955).

*In Russian.

THE IMPORTANCE OF THE PITUITARY-ADRENAL SYSTEM IN THE SENSITIVITY OF ANIMALS TO NARCOTICS AT VARIOUS AGES

I. V. Markova

From the Department of Pharmacology (Head - Corresponding Member AMN SSSR Prof. V. M. Karasik) of the Leningrad Institute of Pediatric Medicine

(Received June 24, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

In a previous paper changes in the sensitivity of rats were described to short-acting (hexobarbital, pentothal), medium (amytal) and long-acting (medinal) barbiturates and to ether; the problem was also discussed of the importance of the comparatively low detoxication of barbiturates in the liver of animals at an early age [2, 3] in the production of the increased sensitivity of these animals to narcotics. It follows from this discussion that the age changes in the detoxicating function of the liver are not of decisive importance.

In the present article the question is examined of the relationship between the increased sensitivity of newborn animals to narcotics and the function of the pituitary-adrenal system. As we know, this system functions very inadequately in newborn animals. Endröczi and Tóth [12], for instance, believe that newborn rats are practically without an adenohypophysis, so that they do not react to different stimuli by the secretion of adrenocorticotropic hormone (ACTH).

Balfour [4] found that calves begin to react to ACTH only on the 10th-40th day after birth. According to Taylor and his co-workers [28], the elaboration and content of ACTH in the hypophysis of children are many (17) times less than in adult human subjects.

Corresponding to the failure of elaboration of ACTH there is a failure of development of the adrenal cortex. In newborn animals this is known to account for 80% of the "fetal" layer [6, 7]; the remaining 20% consists mainly of glomerular and fascicular layers. The latter appear in rats on the 16th-17th day [18], in man in the 3rd-4th months [6, 7] of intrauterine development; in the fascicular layer are produced glucocorticoids [15, 17]. In the first months of postnatal development the "fetal" layer undergoes atrophy and is replaced by the characteristic layers of the adult. The adrenal cortex of newborn animals produces very little corticoids [5-7, 11, 25, 33]. This production reaches the level characteristic of the adult animal at various times depending on the species of animal concerned (in man, for example, at 3-7 years [7]).

The importance of the pituitary-adrenal system for sensitivity to narcotics derives from work which showed that after removal of the adrenals or hypophysis from animals (mice and rats), their sensitivity to narcotics, especially to barbiturates, is sharply increased [10, 19, 24, 29, 31]. As it has been shown [19], the increased sensitivity to barbiturate depends on the fact that in adrenalectomized animals the concentration of barbiturate in the brain is significantly higher than in control animals. This may perhaps be due (or partly due) to the fact that after removal of the adrenals the permeability of the vascular wall increases [20].

After administration of cortisone or oxycortisone to adrenalectomized animals both the concentration of barbiturate in the brain and the duration of narcosis became the same as in control animals (desoxycorticosterone has no such effect). There are also reports that the glucocorticoids, and also ACTH, shorten the period of narcosis even in normal animals [31]. Consequently the cause of the enhanced sensitivity of adrenalectomized animals to narcotics is deficiency of glucocorticoids.

TABLE 1

The Effect of ACTH on the Duration of Hexobarbital and Medinal Narcosis in Rats of Different Ages

Age	Number of animals	Three daily injections of ACTH
1–3 days	60	102±3
1 week	42	101±1,5
2 weeks	32	108±20
3 »	108	75±17
1—1½ months . . .	130	118±15
2 months	40	67±25
3 »	40	52±25

Note: In Tables 1 and 2 the duration of the lateral position in the case of the control animals is taken as 100.

TABLE 2

The Effect of ACTH on the Duration of Hexobarbital Narcosis in Male and Female Rats

Age	Males		Females	
	number of animals	three daily injections	number of animals	three daily injections
3 weeks . .	20	74±19	20	92±15
1 month . .	20	122±15	20	92±6
1½ months . .	20	50±30	20	111±6
2 months . .	20	63±30	20	58±21
3 » . . .	20	37±10	20	87±10

In connection with the above, the hypothesis has been suggested that the increased sensitivity of newborn animals to narcotics depends on the insufficient development of the pituitary-adrenal system. Our investigation was for the purpose of verifying this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on 675 white rats of different ages – from 1 day to 3 months. ACTH (in aqueous solution) was injected intraperitoneally in a dose of 5 units per 100 g body weight, either once or on 2-3 successive days. Control animals were given intraperitoneal injections of the same volume of distilled water. Sucking animals (under the age of 3 weeks) from the same litter were used both in the experiment and as controls. Two hours after the last injection of ACTH the animals were injected subcutaneously in the dorsal region with aqueous solutions of hexobarbital or medinal in doses corresponding to the age of the animals. The length of time in which the animals remained in the lateral position was determined for both the experimental and control group (in the case of the newborn animals this could be taken to mean lying supine). The duration of staying in the lateral position of the control animals was taken as 100 and was compared with the duration of the lateral position in the animals receiving preliminary injections of ACTH.

EXPERIMENTAL RESULTS

In Table 1 are given the results of the experiments in which three daily injections of ACTH were given as being the most demonstrative.

As may be seen from Table 1, the developing animals began to react to ACTH by a shortening of the period of narcosis from the age of 3 weeks, and as their development continued this effect became more and more obvious. However, it should be pointed out that rats of 1-1½ months reacted distinctively to ACTH: injection of ACTH on one or two days had practically no effect on the duration of narcosis, whereas injection for three days lengthened it considerably. This might have been connected with the fact that at this age ACTH stimulates the production of mineralocorticoids the administration of which prolongs narcosis [13, 23, 32]; it may even be used to enhance the effect of luminal and epanutin in the treatment of epilepsy [27].

If the effect of ACTH on the duration of narcosis was analyzed separately in males and females, it appeared that the males began to react much sooner to the drug and that the reaction in them was rather more pronounced (Table 2).

From data in the literature it follows that the functional development of the pituitary-adrenal system in rats terminates at the age of 3 weeks [12].

According to our findings, this was the age at which the young rats began to react to ACTH by a shortening of the period of narcosis. At this time the sensitivity of the young rats became equal to that of the adult animals to pentothal, amytal, medinal and, before long, to ether as well. The results obtained confirmed the hypothesis, mentioned above, that the increased sensitivity of newborn animals to narcotics depends on inadequate development of the pituitary-adrenal system.

This system, however, is not autonomous, but its function is regulated by the central nervous system, in particular by the hypothalamus [9, 14, 34]. As has been shown [1, 8, 16, 21, 22, 30], the central nervous system in rats reaches the characteristic adult level of development at about 2-4 weeks of life. Obviously this is the time at which the central apparatus for regulating the activity of the pituitary-adrenal system begins to function; as a result there is increased production of glucocorticoids and the sensitivity to narcotics becomes reduced.

A high content of glucocorticoids in the body, unaccompanied by the necessary development of the central nervous system cannot affect the sensitivity of the animals to narcotics. It has been shown [26], for instance, that newborn infants have a high content of maternal corticoids (which are very rapidly – by the 5th day – eliminated from the body); despite this, newborn infants are very sensitive to narcotics. The increased sensitivity to narcotics thus depends not so much on lack of glucocorticoids as on failure of development of the hypothalamo-pituitary-adrenal system as a whole.

SUMMARY

Intraperitoneal administration of ACTH shortens the hexenal and medinal narcosis in rats only from the age of 3 weeks. According to the literature data, the development of the pituitary-adrenal system in them is completed by this time. Former observations demonstrated that the sensitivity of rats to pentothal, amytal and medinal at this age (and also to ether when one month old) is equal to the sensitivity of the adult rats. The males begin to react to ACTH by a shortened period of narcosis before the females, and the reaction in the former is more pronounced. These data confirm the assumption that increased sensitivity of newborn animals to narcotics depends on the immaturity of the pituitary-adrenal system.

LITERATURE CITED

[1] E. V. Parin and E. B. Sopotsinskaya, Scientific Papers of the Kharkov University,* vol. 68, pp. 43-49, 1956.

[2] T. N. Reshetnikova, The Action of Barbiturates at an Early Age,* Author's abstract of Dissertation, Tashkent, 1956.

[3] T. N. Reshetnikova, Transactions of the Kirghiz Medical Institute* 8, 213-216, Frunze, 1956.

[4] W. E. Balfour, J. Physiol. 122, 59 (1953).

[5] R. J. Bayliss, Lancet. 1, 62 (1955).

*In Russian.

[6] K. Benirschke, E. Bloch and A. T. Hertig, Endocrinology 58, 598 (1956).

[7] E. Bloch, K. Benirschke and E. Rosemberg, Endocrinology, 58, 626 (1956).

[8] I. Buresh, Zhur. Vysshei Nerv. Deyatel 7, 1, 169 (1957).

[9] J. W. Conn and S. S. Fajans, Ann. Rev. Physiol. 14, 453 (1952).

[10] F. Eichholtz, R. Hotovy and P. Collischonn, Arch. Exper. Path u. Pharmakol. 207, 576 (1949).

[11] A. B. Eisenstein and P. M. Hartroft, Endocrinology 60, 634 (1957).

[12] E. Endröczi and K. Toth, Acta. Physiol Acad. Sc. 8, 1, 33 (1955).

[13] E. Fingl, J. Pharmacol. a. Exper. Therap. 105, 37 (1952).

[14] R. Guillemin, a. Endocrinologe 34, 193 (1957).

[15] Ph. M. Hartroft and A. B. Eisenstein, a. Endocrinologe 60, 641 (1957).

[16] H. E. Himwich and W. A. Himwich, J. Chronic Dis. 3, 487 (1956).

[17] I. Ch. Jones, J. Endocrinol. 9, 38 (1953).

[18] J. B. Josimovich, A. J. Ladman and H. W. Deane, Endocrinology 54, 627 (1954).

[19] A. Komiya and K. Shibata, J. Pharmacol. a . Exper. Therap. 116, 98 (1956).

[20] J. Kramár, V. W. Meyers and H. H. McCarthy, Endocrinology 60, 589 (1957).

[21] P. Mandel, R. Bieth and J. D. Weill, Bull. Soc. Chim. Bio. 37, 475 (1955).

[22] P. Mandel and R. Bieth, The Nucleic Acids. [Russian translation].

[23] J. Mercier, Actualités Pharmacol. 14, 453 (1952).

[24] K. Shibata and A. Komiya, Proc. Soc. Exper. Bio. a. Med. 84, 308 (1953).

[25] M. P. Stack-Dunne and F. G. Young, Ann. Rev. Biochem. 23, 405 (1954).

[26] H. J. Staemmler, Arch. Gynäkol. 182, 521 (1953).

[27] P. H. Staple, J. Endocrinol 9, 18 (1953).

[28] N. R. W. Taylor, J. A. Lorraine and H. A. Robertson, J. Endocrinol. 9, 334 (1953).

[29] H. Waltz, M. Bartels and H. Mathies, Arch. Exper. Pathol. Pharmacol. 224, 523 (1955).

[30] J. D. Weill and P. Mandel, Compt. red. Soc. Bio. 147, 1818 (1953).

[31] Ch. W. Winter and L. Flataker, J. Pharmacol. Exper. Therap. 105, 358 (1952).

[32] D. M. Woodbury, J. Pharmacol. Exper. Therap. 105, 46 (1952).

[33] H. Zeisel, Biologiya 17, 7590 (1957).

[34] S. Zuckerman, Lancet 1, 789 (1954).

CHANGES IN THE INTEROCEPTIVE REFLEXES IN DOGS UNDER THE INFLUENCE OF NARCOTICS

T. V. Popova

From the Laboratory of Pathological Physiology (Head – Prof. V. S. Galkin)* of the I. P. Pavlov Institute of Physiology (Director – Academician K. M. Bykov) of the AN SSSR, Leningrad

(Received July 10, 1958. Presented by Academician K. M. Bykov)

The aim of the present investigation was to study the interoceptive reflexes in dogs without preliminary anesthesia or decerebration.

The action of anesthetics was investigated by the administration of various doses ranging from small amounts causing no change in the animal's behavior to doses causing a state of profound narcosis.

EXPERIMENTAL METHOD

Experiments were carried out on dogs trained to a procedure enabling the recording of the blood pressure without anesthesia.

Interoceptive reflexes were induced by stimulation of the mechanoreceptors of the stomach and rectum, and of the clamped carotid artery brought near the surface in a skin flap (for a description of the method, see papers [9, 10]).

In the experiment various anesthetics were used in the form of a 2.5% aqueous solution: hexobarbital in doses of 0.01-0.134 g/kg, evipan sodium – 0.005-0.116 g/kg, sodium amytal – 0.005-0.09 g/kg, chloralose – 0.005-0.15 g/kg (1% aqueous solution), chloral hydrate – 0.03-0.5 g/kg (10% solution in physiological saline) and urethane – 0.166-1.72 g/kg (20% aqueous solution); the dose of ether was measured at the time of the experiment from the amount actually consumed (before the experiments the animals were given 0.005 g/kg of morphine subcutaneously).

All the anesthetics (except ether) were injected intravenously after a recording had been made of the initial interoceptive reflexes.

In all, 67 experiments were carried out on 11 dogs.

EXPERIMENTAL RESULTS

As a result of the administration of the anesthetics mentioned above, phasic changes were observed in the pressor interoceptive reflexes of the animal.

After small doses or during slow (fractionated) anesthesia an increase in the pressor reflexes and a reduction in the threshold of stimulation could be observed (especially after stimulation of the mechanoreceptors of the rectum). This phase of the change in the reflexes was observed in the absence of any form of alteration in the animal's behavior, was often difficult to detect and, as a rule, was transient. With the onset of sleep or at an earlier period of the action of the anesthetics the second phase of change of the reflexes was observed – their decrease and depression.

* Deceased.

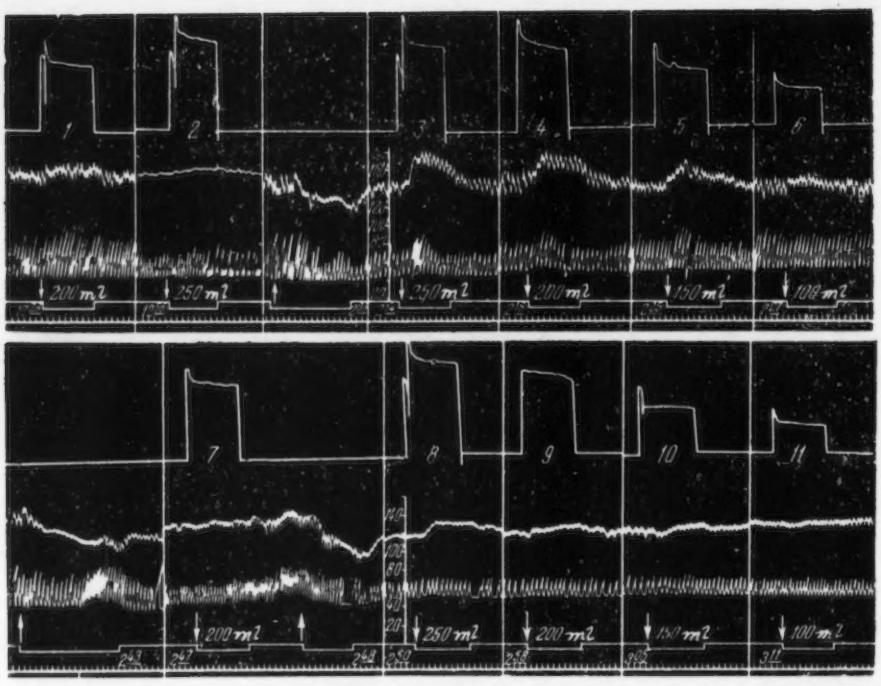


Fig. 1. Changes in the interoceptive reflexes during fractionated intravenous injection of evipan sodium (dog Dzhanka, experiment dated February 11, 1956).
Significance of the curves (from above down): pressure within the organ, arterial pressure, respiration, zero line of the arterial pressure (also the stimulus marker), time marker (5 seconds). 6 and 11) Reflex in response to injection of 100 ml of air into the rectum (↓); 5 and 10) the same in response to 150 ml; 1, 4, 7, 9) the same to injection of 20 ml; 2, 3, 8) the same to injection of 250 ml; ↑) injection of evipan sodium (12, 24 and 14 mg/kg).

When an anesthetic dose was given continuously the phase of increased interoceptive reflexes died out, and a considerable decrease or total suppression of the pressor reflexes was then at once observed.

In several cases depressor changes in the blood pressure were observed, and these usually increased in magnitude with further doses of anesthetic. As the depth of anesthesia lessened, the depressor changes in blood pressure decreased and the pressor reactions were subsequently restored.

Examples of the experiments in which the phasic changes in the pressor reflexes during anesthesia took place as described may be seen on the accompanying kymograms.

During fractional injections of evipan sodium (Fig. 1), for instance, after a dose of 12 mg/ kg a considerable increase of the pressor reflexes was observed in response to stimulation of the mechanoreceptors of the rectum and a fall in the thresholds of stimulation (well marked by comparison of tracings 1 and 2 with 3 and 4). Furthermore reflexes appeared also in response to subthreshold stimulation (injection of 150 and 100 ml of air, tracings 5 and 6). Further addition of evipan sodium lowered the increased pressor reactions.

The extinction of the first phase and suppression of the pressor reflexes during continuous injection of an anesthetic dose of sodium amytal (46 mg/ kg) are shown in Fig. 2, in which the depressor changes in the blood pressure in response to inflation of the stomach (tracing 8), appearing instead of the pressor reflex (tracing 1) are seen to be not sharply pronounced. With deeper anesthesia (for example with a dose of 0.134 g/ kg of hexobarbital) depressor reactions were observed with a significant fall in the blood pressure. The carotid sinus pressor reflex was also considerably decreased in the period of narcotic sleep, and there was observed in addition a

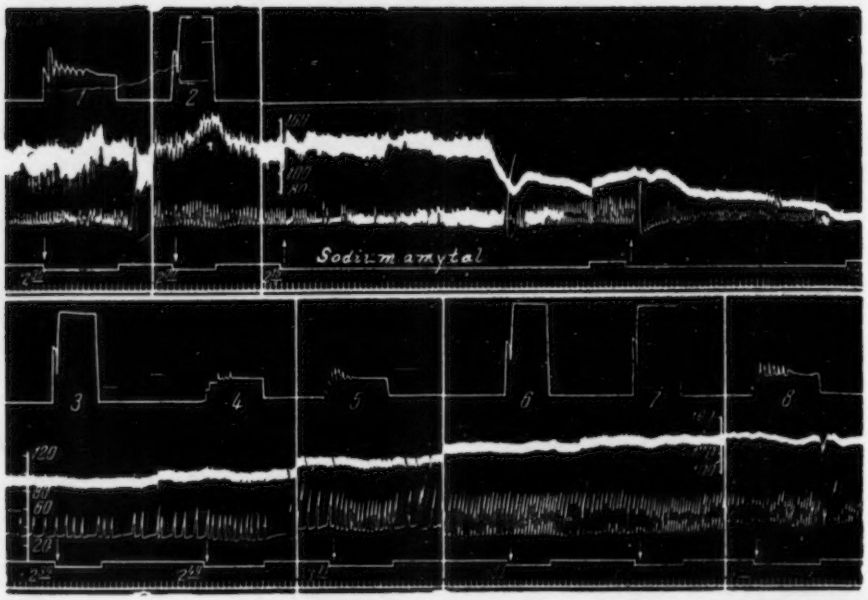


Fig. 2. Changes in the interoceptive reflexes in response to intravenous injection of an anesthetic dose of sodium amytal (the dog Dzhanka, experiment dated May 24, 1956). Significance of the curves as in Fig. 1. 1,4,5,8) Reflexes in response to injection of 1200 ml of air into the stomach (↓); 2, 3, 6, 7) reflexes in response to injectionof 350 ml of air into the rectum; ↑) injection of sodium amytal (46 mg/ kg).

distinctive depressor reaction which appeared after removal of the clamp from the carotid artery.

The results of special control experiments showed convincingly that the changes described in the interoceptive pressor reflexes were not the result of the duration of the experiment nor of repetition of the stimulus.

As an additional control of the results of the investigations the reflexes were tested with changes in the depth of anesthesia, and also experiments were carried out in which various volumes of physiological saline were administered to animals.

As may be seen from Fig. 3, the reflex reactions of the blood pressure in response to repeated interoceptive stimulation in a prolonged control experiment during which physiological saline was given, continued without appreciable change.

The standard pattern of the phasic changes in the interoceptive reflexes during anesthesia indicated that, in principle, the action of all the anesthetics used was the same.

If, however, the doses causing the first and second phases of the changes in the pressor reflexes were compared with the anesthetic doses (in our experimental conditions), a difference was found in the degree of the inhibiting effect of the different anesthetics on the interoceptive reactions which we studied.

The most marked depression of the interoceptive reflexes was caused by sodium amytal and chloral hydrate. The phase of increase of the reflexes and also their subsequent decrease appeared in response to injection of these drugs in doses significantly smaller than the minimal doses having a narcotic effect. Thus even before the onset of sleep the interoceptive reflexes in response to the injection of chloral hydrate and sodium amytal were, as a rule, considerably reduced.

Injection of chloralose also reduced the reflexes even before injection of an anesthetic dose. However, in the first phase of action the pressor reflexes were increased to a greater extent (than by sodium amytal and chloral hydrate), although this period was also quite short in duration.

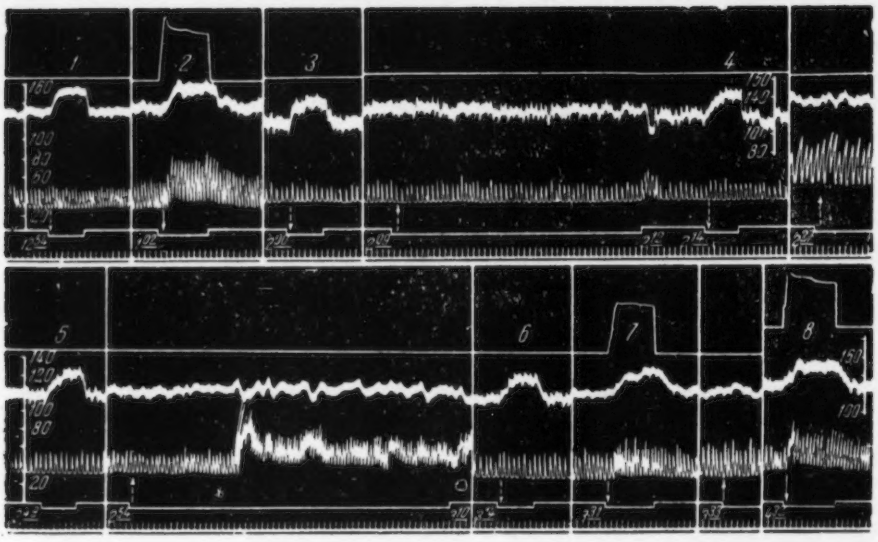


Fig. 3. Interoceptive reflexes in a control experiment (in response to the intravenous injection of physiological saline (the dog Pamir, experiment dated October 18, 1956). Significance of the curves as in Fig. 1. 1,3,4,5,6) Reflexes in response to clamping the carotid artery (↓); 2, 7, 8) reflexes in response to injection of 200 ml of air into the rectum (↓); ↑) injection of physiological saline (20, 20, 40 and 20 ml).

In contrast to these narcotics, hexobarbital and evipan sodium reduced the reflexes only after sleep had been induced in the animal. Furthermore, in some experiments in which hexobarbital was injected in fractional doses, increased reflex reactions could be observed even in the period of profound sleep.

In the experiments with ether an obvious and prolonged increase took place in the interoceptive reflexes, in some cases maintained throughout the whole experiment in spite of the adequate depth of the ether anesthesia, which was especially marked after preliminary depression of the reactions by administration of morphine.

Urethane occupied a special place among the anesthetics. After the phase of increase of the interoceptive reflexes, with increasing depth of urethane anesthesia by fractional injection of the drug (between phases I and II) a period ensued of normalization of the reflexes, which corresponded to the end of the period of excitation and the beginning of light anesthetic sleep (in a dose of 73-109% of the anesthetic dose).

Following preliminary inhalation of ether this period occurred later, at a dose equivalent to 100-160% of the minimum anesthetic dose (under these conditions of anesthesia). This is an important factor because such a level of anesthesia is in practice adequate for the performance of acute experiments and also in view of the more common usage of ether-urethane anesthesia in experimental practice.

On testing the other anesthetics we observed an intermediate phase of normalization of reflexes only in the experiments in which fractional injections of chloralose were given. However, this period was very transient and appeared in response to a dose equivalent to 43-57% of the anesthetic dose, i.e. before the animal fell asleep.

Hence, in light anesthetic sleep, with the exception of sleep induced by ether, urethane, and sometimes, hexobarbital, we always observed decreased interoceptive reflexes.

A particularly pronounced depressing effect on the interoceptive reflexes was shown by narcotics which are widely used in clinical practice for therapeutic purpose (sodium amytal and chloral hydrate).

On the bais of the results obtained we cannot agree with Gero's view [3] that the therapeutic action of narcotic drugs (in sleep therapy) is effected in a period of increase of interoceptive reflexes.

This hypothesis is unlikely, since the phase of increase of the interoceptive reflexes is very brief and (in

particular for chloral hydrate and sodium amytal) it is observed only at the very beginning of administration of the narcotics, at a dose which is till insufficient to have a hypnotic action.

Furthermore, clinical and experimental observations have shown that sleep therapy is effective only in those cases when it is essential to produce protective inhibition, in contrast to states when it is important to activate the protective functions of the body, since the latter are depressed by narcotics [2].

These observations also confirm the fact that the phase of increase of reflex reactions, if it arises at all, is less important during narcotic sleep than the depression of reflexes, which is predominant under such conditions.

The view that a temporary suppression of the interoceptive reflexes may be of therapeutic importance in certain diseases is held at the present time by clinicians and also has experimental grounds in its favor [12, 1, 4, 8, 5, 7, 6, 10, 11 etc.].

SUMMARY

Experiments were performed on dogs without their preliminary narcotization. The author studied the effect of various anesthetics on the reflex changes of the blood pressure occurring in interoceptive stimulation. Phasic changes were noted in interoceptive reflexes with the increase of the doses of anesthetics (at first they increased and later decreased, became depressed and inversed).

In fractional administration of urethane (especially after the preliminary inhalation of ether) the period of normalization of the reflexes was noted. By this urethane differs from other anesthetics.

Is is assumed that the depressing effect of the anesthetic on the interoceptive reflexes (especially in the administration of chloral hydrate and sodium amytal) plays the main role in the mechanism of sleep therapy in clinical conditions.

LITERATURE CITED

[1] K. M. Bykov, cited by Kulikova, Pshonik and Segal'.

[2] V. S. Galkin, Anesthesia (New Experimental Findings), Kirov (1944).*

[3] Ya. Gero, Chekhoslovatskaya Fiziologiya 4, No. 1, 66-75 (1955).

[4] Gilyarovskii, cited by Kulikova, Pshonik and Segal'.

[5] G. S. Kan, Problems of the Physiology of Interoception, 1, 212-223, 224-231, Moscow – Leningrad (1952).*

[6] G. S. Kan, Proceedings of the Second All-Union Conference of Pathophysiologists, 154-155, Kiev (1956).*

[7] Yu. G. Kozlov, The Pathophysiological Mechanisms of Hypochondriac Delirium, Candidate's Dissertation, Leningrad (1954).*

[8] V. S. Kulikova, A. T. Pshonik and E. Yu. Segal', Problems of Cortico-Visceral Pathology, 289-300, Moscow – Leningrad (1952).*

[9] T. V. Popova, Byull. Éksptl. Biol. i Med. 39, No. 6, 32-36 (1955).

[10] T. V. Popova, Byull. Éksptl. Biol. i Med., 42, No. 7, 33-38 (1956).**

[11] T. V. Popova, Byull. Éksptl. Biol. i Med., 43, No. 5, 71-77 (1957).**

[12] V. N. Chernigovskii, Transactions of the Naval Medical Academy, vol. 17, 395-437, Leningrad (1949).

*In Russian.

**Original Russian pagination. See C. B. Translation.

IMPULSES IN THE SENSORY NERVES IN RESPONSE TO STIMULATION OF THE SKIN RECEPTORS OF IMMUNIZED ANIMALS WITH ANTIGEN

A. N. Gordienko, B. A. Saakov and I. M. Bondarev

From the Department of Pathophysiology (Head – Prof. A. N. Gordienko) of the Rostov Medical Institute

(Received January 29, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

We showed in a previous paper [1] that the intradermal injection of antigen causes stimulation of receptors, accompanied by a characteristic flow of impulses along a sensory nerve.

We know that the immunized animal reacts differently from the unimmunized by the production of antibodies in response to a repeated injection of antigen. On repeated injection of antigen into immunized animals a rapid and high increase in the antibody titer takes place. In unimmunized animals the injection of the same dose of antigen usually causes a slow and slight increase in the antibody titer.

Believing that the production of antibodies has a reflex mechanism, and that the impulses in the nerve may be one of the factors responsible for the formation of antibodies, we attempted to study the reaction of a sensory nerve of an immunized animal to the intradermal injection of antigen.

EXPERIMENTAL METHOD

Experiments were carried out on dogs immunized by triple injection of typhoid vaccine. The animals took part in the experiment 7-10 days after injection of the last dose of vaccine.

The flow of impulses in sensory nerves (auricular and saphenous nerves) was studied before and after the intradermal injection of typhoid antigen into the region innervated by the corresponding nerve.

In control experiments dysentery antigen was injected. The nerve to be examined was placed on a recording platinum electrode (distance between electrodes 4 mm), the potentials were fed into an amplifier (type 2-cube-2) and recorded on a loop oscillograph.

After the initial flow of impulses had been recorded the antigen was injected and the changes in the flow of impulses recorded immediately after the injection or 1, 3 and 5 minutes later.

EXPERIMENTAL RESULTS

In 17 dogs immunized with typhoid vaccine it was shown that the intradermal injection of typhoid bacillus antigen caused a significantly greater reaction than in unimmunized animals.

Two types of reaction were observed. In the first case, intradermal injection of typhoid bacillus antigen caused a slow change of the bioelectric potentials. The electroneurogram showed the appearance of separate, biphasic spike oscillations, after which the frequency of the potential variations increased and sometimes acquired the character of grouped impulses.

As a rule after 15-20 minutes the bioelectrical activity of the nerve increased sharply and changed into

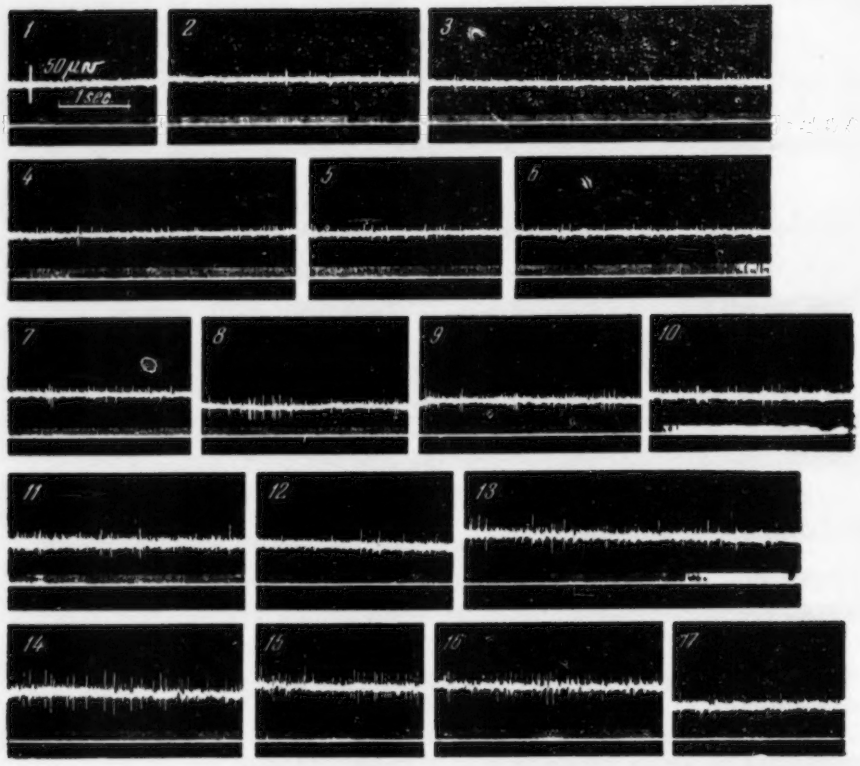


Fig. 1. Changes in the action potentials of the saphenous nerve of an immunized dog after intradermal injection of typhoid bacillus antigen (first type of reaction). Tracing 1) initial flow of impulses; 2-17) tracing made after 30 seconds, and 1, 3, 5, 7, 10, 15, 17, 20, 25, 30, 35, 40, 50, 60 and 75 minutes respectively after injection of antigen.

a continuous flow of impulses. This reaction was comparatively prolonged and was recorded for 70-80 minutes.

In unimmunized animals the same vaccine caused an identical flow of impulses but the reaction did not last longer than 7-8 minutes.

In the electroneurograms of experiment No. 6 (Figure 1), by comparison with the initial flow of impulses [1], changes were seen, which were not most pronounced 10 minutes [7] after injection of the antigen and which lasted for an hour [8-16].

The intradermal injection of typhoid antigen into immunized animals thus caused a more prolonged reaction than took place in the unimmunized animals.

In the second case, immediately after the injection of typhoid antigen, the immunized animals developed a well-marked flow of impulses, although the duration of this reaction was considerably less than in the first group of animals.

In Fig. 2 are shown extracts of electroneurograms recorded before and 1, 3, and 5 minutes after injection of the antigen, from which it can be seen that changes in the initial activity [1] developed immediately after injection of the antigen, and the excitation which ensued continued to be recorded for 15 minutes [7].

Both types of reaction were characterized by a more prolonged flow of impulses than occurred during the changes in bioelectrical activity of the same sensory nerves in response to injection of the same antigen into intact animals.

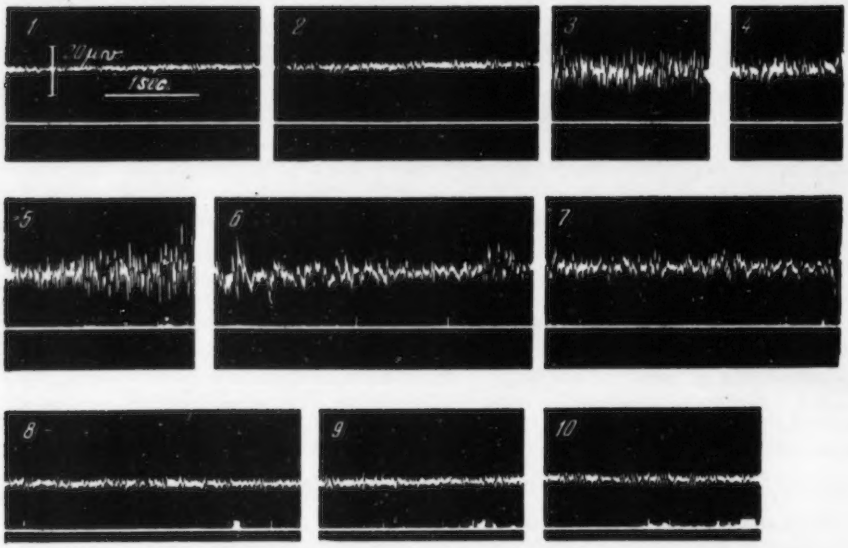


Fig. 2. Changes in the action potentials of the saphenous nerve after intradermal injection of typhoid antigen (second type of reaction).
Tracing 1) initial flow of impulses; tracing 2-10) made 30 seconds and 1, 3, 5, 10, 15, 17, 20 and 25 minutes respectively after injection of the antigen.

The high intensity and the long duration of the reaction was probably one cause of the more intensive production of antibodies in the immunized than the unimmunized animals.

Preliminary immunization of the animals evidently increased the excitation of the receptors toward the corresponding antigens, so that they caused a more intensive stimulation. The strong stimulation of the receptors

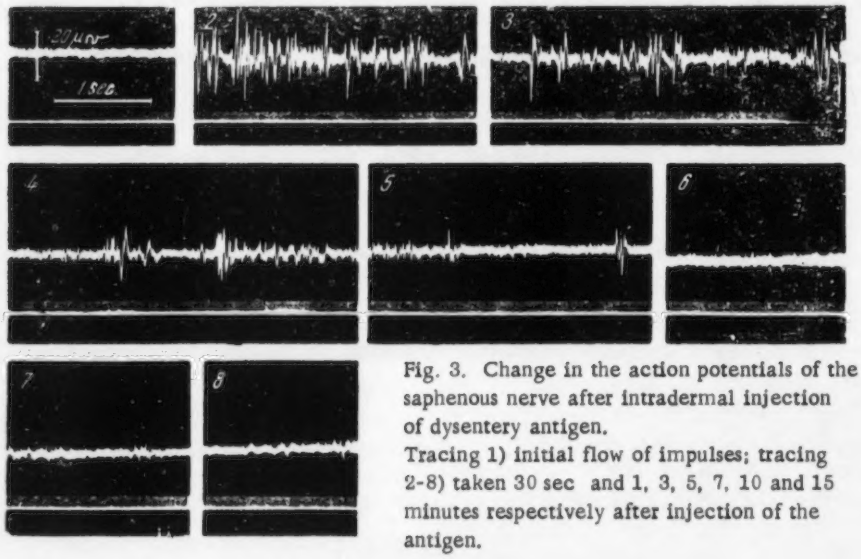


Fig. 3. Change in the action potentials of the saphenous nerve after intradermal injection of dysentery antigen.
Tracing 1) initial flow of impulses; tracing 2-8) taken 30 sec and 1, 3, 5, 7, 10 and 15 minutes respectively after injection of the antigen.

and the weaker adaptation of the receptors towards the antigen were responsible for the intensive production of antibodies in the immunized animals. The increase in this reaction demonstrated that the adaptation of the animal

proceeded along the lines of intensification of the reaction of the nervous reflex apparatus to antigenic stimulation.

The question arose: to what extent was this reaction specific?

In order to answer this question, control experiments were set up. Dogs were immunized with typhoid vaccine and given injections of dysentery bacilli, after which an ordinary reaction was observed in the sensory nerve, whose duration did not exceed the limits which we had found in unimmunized animals [1]. The reaction lasted as a rule for 5-7 minutes (Fig. 3).

It can be seen in this figure that in response to injection of dysentery antigen, the characteristics of amplitude and frequency of the reaction differed from those observed in the main experiments (see Figs. 1 and 2). It must be pointed out that this reaction was similar in its nature to that observed in the unimmunized animals.

The data given demonstrate that in the process of immunization of animals a change occurs in the specific sensitivity of the receptor apparatus of the skin. The skin receptors of immunized animals react for a longer period of time to specific antigens.

This intensification of the reaction is probably one of the more important factors determining the changed reaction of immunized animals to injection of antigen.

The change in the reaction demonstrates once again the an immunogenesis is brought about primarily by stimulation of the nervous system and of its receptor apparatus.

It may be thought that in the process of immunization changes occur in the reactivity not only of the skin receptors but also of other components of the reflex apparatus. A study of the functional changes in the other links of the reflex apparatus during immunization is of great interest and will be the subject of our later research.

SUMMARY

The skin receptors of the immunized animals react to specific antigens for a more prolonged time and often with greater intensity. The reaction is more prolonged, the frequency of the bioelectrical potential oscillations of the sensory nerves becomes greater. This is, probably, one of the most important factors showing the changed reaction of the immunized animals to the administration of the antigen.

LITERATURE CITED

[1] A. N. Gordienko, V. I. Kiseleva, B. A. Saakov et al., Byull. Éksptl. Biol. i Med., Supplement to No. 1, 147-151 (1957).

ONCOLOGY

A COMPARATIVE STUDY OF THE SEROLOGICAL PROPERTIES OF FRACTIONS OF A CANCER ANTISERUM FROM HORSES

N. A. Nazarenko and V. S. Gostev

From the Laboratory of Immunochemistry (Head - Prof. V. S. Gostev) of the Institute of Experimental Biology (Director - Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received July 4, 1958. Presented by Active Member of the AMN SSSR N. N Zhukov-Verezhnikov)

It is now an accepted fact that antibodies are firmly combined with those serum proteins which have a high molecular weight, a large particle size, and a low electrical mobility, i.e. with the γ-globulins.

It is impossible to separate antibodies from the serum γ-globulins by precipitation [2, 11, 12, 14], ultrafiltration [10, 13, 17], ultracentrifugation [8, 15] or electrophoresis [16].

The proof of the γ-globulin nature of antibody has led to the use of the γ-globulin fraction of the serum instead of whole serum as a therapeutic preparation.

N. V. Kholchev and L. I. Kolesnikova [6], for instance, developed a commercial method of obtaining an antimeasles γ-globulin by precipitating it with alcohol in the cold. The use of this preparation showed that 1.5-3.0 ml of it was equivalent in its effect to 30-60 ml of the whole serum.

At the I. I. Mechnikov Moscow Institute of Vaccines and Sera a method has been developed for the production on a commercial scale of 15 types of therapeutic antisera against various infectious diseases. These preparations do not produce in animals any of the temperature reactions or sensitization phenomena usually observed after administration of whole serum [4].

Undoubted interest is being shown in the fractionation of cytotoxic sera, with the removal of ballast proteins from the sera and separation of the fraction bearing the cytotoxic properties in the form of a homogeneous preparation of maximum purity.

The association between the cytotoxin and the globulins has now been proved. It has been shown, for example, that the serologically active fraction of the antireticuloendothelial cytotoxic serum (ACS) obtained from rabbits was the β-globulin, and of the ACS obtained from horses it was the γ-globulin [5].

By far the majority of authors believe that the antibodies of the rabbit serum are combined with the γ-globulin fraction of the antiserum. This was very convincingly shown by Tiselius and Kabat [16], by means of a combination of electrophoretic and serological analysis of the serum of hyperimmunized rabbits.

At the present time, however, many workers are increasingly suggesting the possible combination of antibodies with the other fractions, and in particular, with the β-globulin fraction. The problem of which fraction of the cancer antiserum obtained from the horse has the serological activity concentrated in it has received little study.

In pilot experiments by our laboratory colleague Yu. V. Zykov (1957), it was shown that the γ-globulin of a cancer antiserum from the horse contained the main serological antibody activity.

TABLE 1

The Classic CFT with Fractions of Cancer Antiserum No. 2 from Horses (experiment dated November 10, 1957)

Serum fraction	Antigens	Intensity of reaction in relation to quantity of protein (in mg) taken for experiment									Antigen control
		2,6	1,3	0,65	0,32	0,26	0,13	0,065	0,032	0,016	
γ-globulins	Tissue from carcinoma of the stomach	–	–	–	–	++++	++++	+++	+	h	h
	Normal stomach tissue	–	–	–	–	+	h	h	h	h	h
	Spleen tissue	–	–	–	–	+	h	h	h	h	h
γ-globulin control		–	–	–	–	h	h	h	h	h	h
α_1-α_2- and β-globulins	Tissue from carcinoma of the stomach	2+	+	h	h	h	h	h	h	h	h
	Normal stomach tissue	+	h	h	h	h	h	h	h	h	h
	Spleen tissue	±	h	h	h	h	h	h	h	h	h
α_1-α_2- and β-globulin control		h	h	h	h	h	h	h	h	h	h
Albumins	Tissue from carcinoma of the stomach	h	h	h	h	h	h	h	h	h	h
	Normal stomach tissue	h	h	h	h	h	h	h	h	h	h
	Spleen tissue	h	h	h	h	h	h	h	h	h	h
Albumin control		h	h	h	h	h	h	h	h	h	h

Note: – indicates anticomplementary effect of γ-globulins; h) hemolysis.

The purpose of the present research was to obtain a purified, concentrated anticancer preparation by fractionation of a cancer antiserum from horses, and to study the immunological features of the fractions obtained.

EXPERIMENTAL METHOD

Fractionation was carried out on a cancer antiserum of high specificity, obtained from horses for therapeutic purposes in our own laboratory by Prof. V. S. Gostev, Yu. V. Zykov and A. A. Saakov [3].*

The antiserum was fractionated by the method of cold precipitation with methanol [9]; three fractions were obtained: γ-globulins, α_1-, α_2- and β-globulins and albumins. The purity of the fractions was tested by paper electrophoresis. The fractions obtained were examined by the classic complement fixation test (CFT) and by the quantitative CFT to 50% hemolysis end-point, with both soluble and adsorbed [7] antigens.

The test antigens used were saline extracts of human normal and tumor tissue prepared in a 0.85% solution of NaCl in a proportion of 1:10. The adsorbed test antigens were prepared by adsorption of these extracts on chromatographic paper, by a method previously described by us [1].

* Immunization of the horses and preparation of the sera were undertaken at the Gamaleya Institute of Epidemiology and Microbiology, under the direction of G. E. Ryabkov and A. V. Beilinson.

TABLE 2

Quantitative CFT to 50% Hemolysis End-Point with Cancer Antiserum from the Horse and with Its Fractions (test antigen – saline extract of tissue)

Test antigens	Antigen dilutions	Serum No. 2 dated November 10, 1957, original		γ-Globulins		α_1-α_2- and β-globulins		Albumin		Antigen controls	
		units of complement									
		free	fixed	free	fixed	free	fixed	free	fixed	free	fixed
Human gastric carcinoma tissue	1:10	—	43,7	—	43,7	3,6	40,1	43,7	—	43,7	—
	$1:10^2$	—	43,7	—	43,7	4,6	39,1	43,7	—	43,7	—
	$1:10^3$	4,6	39,1	—	43,7	43,7	—	43,7	—	43,7	—
	$1:10^4$	4,8	38,9	4,1	39,6	43,7	—	43,7	—	43,7	—
Normal human stomach tissue	1:10	—	43,7	—	43,7	43,7	—	43,7	—	43,7	—
	$1:10^2$	3,4	40,3	—	43,7	43,7	—	43,7	—	43,7	—
	$1:10^3$	4,8	38,9	3,7	40,0	43,7	—	43,7	—	43,7	—
	$1:10^4$	43,7	—	4,5	39,2	43,7	—	43,7	—	43,7	—
Human spleen tissue	1:10	3,4	40,3	3,4	40,3	43,7	—	43,7	—	43,7	—
	$1:10^2$	3	40,7	3,4	40,3	43,7	—	43,7	—	43,7	—
	$1:10^3$	43,7	—	3,7	40,0	43,7	—	43,7	—	43,7	—
	$1:10^4$	43,7	—	4,6	39,1	43,7	—	43,7	—	43,7	—
Serum controls	1:10	43,7	—	43,7	—	43,7	—	43,7	—	43,7	—
Complement control	Undiluted	43,7	—	—	—	—	—	—	—	—	—

TABLE 3

Quantitative CFT to 50% Hemolysis End-Point with Fractions of Cancer Antiserum from the Horse (antigen – saline extract adsorbed on paper)

Test antigens	γ-Globulins		α_1-α_2- and β-globulins		Albumins		Antigen control	
	units of complement							
	free	fixed	free	fixed	free	fixed	free	fixed
Gastric carcinoma tissue	—	47,5	3,5	44,0	47,5	—	47,5	—
Normal stomach tissue	—	47,5	3,1	44,4	47,5	—	47,5	—
Serum control	47,5	—	47,5	—	47,5	—	47,5	—
Complement control	—	—	—	—	—	—	—	—

EXPERIMENTAL RESULTS

Table 1 shows the results of a typical classic CFT with the fractions of the cancer antiserum from horses – γ-globulins, α_1-, α_2- and β-globulins and albumins. The quantity of the fractions taken for the experiment was estimated in accordance with their protein content. As test antigens we used saline extracts of tissue from a carcinoma of the stomach, of normal stomach tissue and of spleen tissue from human subjects. Complete hemolysis was observed in the control experiments to check the antigens, the fractions and the complement. In view of the high anticomplementary effect of the γ-globulin, the test was only valid starting with the samples containing 260 γ of protein.

The serological activity of cancer antiserum from the horse, as determined by the classic CFT, was concentrated mainly in the γ-globulin fraction; the α_1-, α_2- and β-globulin fractions were considerably less active. No antibodies were found in the albumins.

Analagous results were also obtained by another method – the quantitative CFT to a 50% hemolysis end-point with soluble antigens. The test antigens (saline extracts of tissue from carcinoma of the stomach, normal stomach tissue and spleen tissue from human subjects) were used in dilutions of 1:10, $1:10^2$, $1:10^3$ and $1:10^4$. The antiserum and its fractions were used in a dilution of 1:10.

As shown in Table 2, the original serum reacts with human gastric carcinoma tissue in an antigen dilution of $1:10^4$, fixing 38.9 units of complement of a total of 43.7 units taken in the experiment; with normal stomach tissue the reaction was weaker: in an antigen dilution of 10^4 there was no reaction, and with spleen tissue there was no reaction at an antigen dilution of $1:10^3$.

The serological activity of the γ-globulin fraction was significantly higher than that of the original serum. The number of units of complement fixed by antigens from gastric carcinoma tissue, from normal stomach and spleen tissue in a dilution of $1:10^4$ was greater than with the original serum. The serological activity of the α_1-, α_2- and β-globulin fractions was sharply reduced. The reaction took place only at the first two antigen dilutions of human gastric carcinoma tissue. No antibodies could be found in the albumins.

The γ-globulin fraction, therefore, is a fraction of high serological activity, in which is concentrated almost all the antibodies which are specific toward cancer and normal tissues in man. So far as the α_1-, α_2- and β-globulin fractions were concerned, they were active only in relation to human gastric carcinoma tissue. The albumins were serologically inactive.

Analagous results were obtained by the CFT to a 50% hemolysis end-point. For this test, 20 mg of adsorbed test antigens was usually taken (human gastric carcinoma tissue and normal human stomach tissue).

The antiserum fractions used in the tests were diluted 1:10.

It can be seen from the results shown in Table 3 that the γ-globulin fraction of the cancer antiserum from the horse also revealed the highest serological activity in the reaction with adsorbed test antigens, by specifically

fixing complement. Gastric carcinoma tissue, like normal stomach tissue, fixed all 47.5 units of complement used in the test; a somewhat weaker reaction was given with these antigens by the α_1-, α_2- and β-globulin fractions. The albumins were serologically inactive.

It follows from the experimental findings that the antibodies in the cancer antiserum of the horse were concentrated mainly in the γ-globulin fraction. So far as the albumin fraction is concerned, from the immunological point of view this could be regarded as ballast protein.

Hence in the preparation of a concentrated, purified anticancer preparation, the albumins should be removed.

On the basis of our findings, under the direction of N. A. Ponomareva in the laboratory of the I. I. Mechnikov Institute, an anticancer preparation was produced by alcohol precipitation from a large volume of cancer antiserum of high specificity, and consisted of the γ-globulin fraction of cancer antiserum from the horse.

The serological activity of the anticancer preparation was expressed in units of activity towards cancerous and normal human tissues.

In our opinion the unit of serological activity should be taken as the minimum quantity of the anticancer preparation (in mg of protein as nitrogen) reacting with the corresponding test antigen, adsorbed on paper, in the CFT to 50% hemolysis end-point.

Sorbed antigens in the dry form retain their original serological activity for many months, which ensures standard conditions of determination of the activity of the preparations. This is also facilitated by the conditions of performance of the quantitative CFT to 50% hemolysis end-point.

We have determined the number of anticancer units (ACU) and antinormal tissue units (ANU) calculated per ml of the preparation. For this purpose the preparation was titrated in successive dilutions to estimate the minimum quantity of protein taking part in the reaction with the corresponding adsorbed test antigens. This minimum quantity of protein in the preparation was taken as 1 unit of serological activity. In order to determine the number of units of activity in a given preparation, the total number of mg of protein per ml of the preparation must be divided by the number of mg of protein equivalent to one unit of activity.

Example. Number of mg of protein in the original preparation – 80
1 ACU – 0.6 mg
1 ANU – 6.0 mg
80: 0.6 – 133.3 ACU
80: 6.0 – 13.3 ANU

SUMMARY

As a result of the comparative study of the serological properties of the various fractions of the anticancer serum of the horse by different methods, it has thus been shown that the serological activity is concentrated mainly in the γ-globulin fraction and, to some extent, is present also in the α_1-, α_2 and β-globulin fractions, and this must be taken into consideration during the production of purified, concentrated preparations. The method of determination of the serological activity of an anticancer preparation which we have developed expresses this as units of activity towards malignant and normal human tissues.

LITERATURE CITED

[1] V. S. Gostev and N. A. Shagunova, Byull. Éksptl. Biol. i Med. 10, 121-125 (1957).*

[2] S. K. Dzerzhgovskii and S. N. Predtechenskii, Arkh. Biol. Nauk SSSR. 14, 1-2, 163-185 (1908).

[3] Yu. V. Zykov, Proceedings of a Conference of Young Scientists of the Institute of Experimental Biology of the AMN SSSR, pp. 9-10, Moscow, 1958.**

[4] N. A. Ponomareva, G. I. Babaeva and M. N. Durasova, Theses Commemorating the 35th Anniversary of the Mechnikov Moscow Institute of Vaccines and Sera, 1955.*

[5] Yu. V. Rapp, Cytotoxins in Modern Medicine, 270-274, Kiev, 1956.**

*Original Russian pagination. See C. B. Translation.
**In Russian.

[6] N. V Kholchev and L I Kolesnikova, Zhur. Mikrobiol., Epidemiol. i Immunobiol., No. 5, 6-12 (1947).

[7] N. A Shagunova, Byull. Eksptl. Biol. i Med. 3, 122-127 (1958).*

[8] J. Biscoe, F. Herivick and R. Wyckoff, Science 83, 602-603 (1936).

[9] J. M. Dubert, P. Slizewicz, P. Rebeyrotte and M. Machebocuf, Ann Inst. Pastur. 84, 370-375 (1953).

[10] W. Elford, P. Grabov and W. Fischer, Bioch. Z. 30, 92-99 (1936).

[11] L. Felton, J. Imm. 21, 357-373 (1931).

[12] L. Felton, J. Imm. 22, 453-467 (1932).

[13] K. Goodner, F. Horsfall and I. Bauer, Proc. Soc. Exp. Biol. and Med. 34, 617 (1936).

[14] P. Murdick and S. Cohen, J. Imm. 29, 205-208 (1935).

[15] E. Smith and D. Brown, J. Biol. Chem. 183, 1, 241-249 (1950).

[16] A. Tiselius and E. Kabat, J. Exper. Med. 69, 119-131 (1939).

[17] S. Went and L. Sarkady, Z. Imfrschg. 91, 157 (1937).

*Original Russian pagination. See C. B. Translation.

EXPERIMENTAL BIOLOGY

MORPHOLOGICAL CHANGES IN THE JEJUNUM AND ILEUM OF RATS AFTER WIDE RESECTION OF THE SMALL INTESTINE

N. P. Bochkov

From the Laboratory of Growth and Development (Head - Prof. L. D. Liozner) of the Institute of Experimental Biology (Director - Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received March 3, 1958. Presented by Active Member of the AMN SSSR N. N. Zhukov-Verezhnikov)

Information in the literature on the subject of the character of the morphological changes taking place in the mucous membrane of the area of the intestine left behind after resection, is controversial. Albu [5], Trzebicky [12], Diliberti-Herbin [7] and Schlatter [10], for instance, completely deny the development of any essential morphological changes in the intestine after resection. Other authors [1, 4, 9, 11] believe that thickening of the mucous membrane, dilatation of the lumen of the bowel and hypertrophy of the villi take place in the portion of the intestine remaining after resection. A third group of workers [6, 8] asserts that in addition to processes of hypertrophy, there is an increase in the number of structural units in the intestine (crypts, villi, lymphoid follicles).

All these investigations suffer from an essential technical fault: the changes in the mucous membrane were not compared with the changes in the length and diameter of the intestine. Nevertheless, without at the same time allowing for the length of the intestine, its diameter and the number of villi per unit area, it is impossible to assess correctly either the degree or the character of the morphological changes, since the thickness of the mucous membrane, the dimensions of the villi and their number per unit area depend on the degree of contraction of the muscular coats.

EXPERIMENTAL METHOD

As experimental animals white rats were used. In this way experiments could be carried out on a large number of animals. Furthermore, the relief of the mucous membrane of the intestine is not so complicated in rats as in other mammals. The experiment was performed on 48 animals with an initial weight of 100-140 g (each group consisted of 8-12 animals). At the end of the experiment the rats weighed 200 -300 g. From $^{1}/_{3}$ to $^{1}/_{2}$ of the middle portion of the small intestine was resected from the experimental rats (Table 1).

Animals were killed at various times: 10 days, 1 and 2 months after resection of the intestine. In the first days after operation the weight of the majority of rats fell by 10%. Its restoration was observed by the 7th-10th day. Control rats were killed at the beginning and end of the experiment. The rats underwent operation and were killed at the same time of day, always after starvation for 24 hrs. The following conditions were observed during removal of the intestine for examination. Under ether anesthesia the duodenum was divided from the stomach and the ileum from the large intestine. A cannula was inserted in the proximal end of the intestine, and through it the intestine was perfused with warm physiological saline at a pressure of 25-30 mm of mercury for 2 minutes. At this pressure the intestine is distended to roughly the same extent as by the passage of chyme. The fluid emerges from the ileum in drops. The intestine is washed completely free from food particles. The washing with physiological saline was replaced by perfusion for 5 minutes with Bouin's fluid. At this time the intestine was completely cut away from the mesentery and immersed in fixing solution. After 24 hours the

TABLE 1

Mean Values of the Length, Diameter and Area of the Intestinal Mucosa Before and After Resection of the Intestine

Measurements \ Nature of experiment	Initial control	Final control	Time of operation: 10 days, resected portion	10 days, residual portion	1 month, resected portion	1 month, residual portion	2 months, resected portion	2 months, residual portion
Length of intestine (in cm)	102	129	46	75	35	76	38	83
Circumference of mucous membrane (in cm)	0,85	0,85	0,87	1,31	0,98	1,19	1,02	1,19
Area of mucous membrane (in cm^2)	87	110	40	98	34	90	39	99
Diameter of intestine (in mm)	4,3	4,6	—	5,4	—	5,3	—	5,1

TABLE 2

Average Number of Villi in an Area of 9 mm^2

Part of intestine \ Character of the experiment	Initial control	Final control	Time after operation: 10 days	1 month	2 months
Duodenum	71	38	36	42	48
Middle of the jejunum	103	84	48	71	61
Middle of the ileum	148	133	117	127	97
Distal end of the ileum	212	147	139	153	135

TABLE 3

Mean Thickness of the Crypt-Bearing Layer of the Mucous Membrane in μ, Before and After Resection of the Intestine

Portion of intestine \ Nature of experiment	Initial control	Final control	Time after operation: 10 days	1 month	2 months
Jejunum	83	81	144	134	109
Ileum	99	83	142	129	132

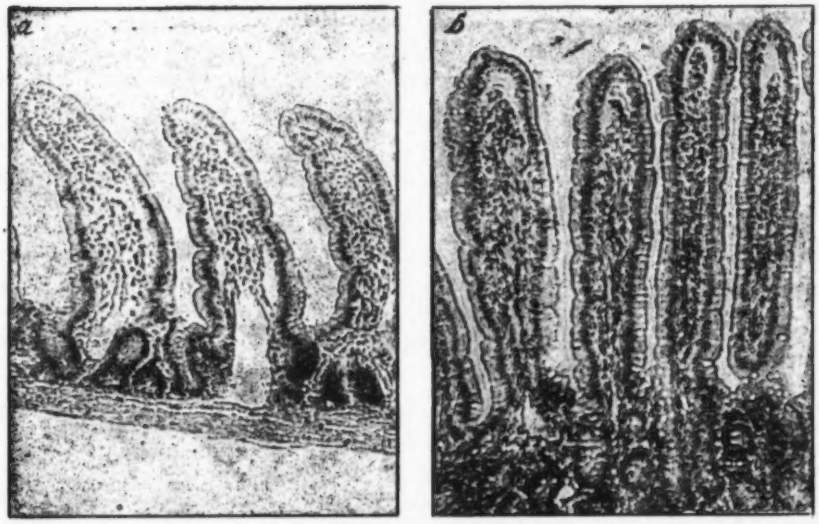
length of the intestine was measured, together with its diameter in 30-40 places (by means of a ruler and millimeter squared paper), the number of lymphoid follicles was counted, and sections were cut for histological examination. The resected area was subjected to the same treatment. Specimens of tissue were taken in the control rats from the middle of the jejunum and ileum, and in experimental animals 5-7 cm proximally and distally to the point of anastomosis. Transverse and longitudinal histological sections were cut to a thickness of 7 μ, and stained with hematoxylin-eosin. The circumference of the mucous membrane was measured at its junction with the submucosal layer in the jejunum and ileum, and the average deduced.

The length and width of the villi were measured (in their middle part), and the number of cells counted in the epithelial layer of the middle part of the villi throughout the diameter of one field of vision with magnification of $10 \times 60 \times 1.5$. By means of a binocular loupe the number of villi was counted in an area of 9 mm^2 of the

TABLE 4

Mean Length and Width of the Villi in μ Before and After Resection of the Intestine

Nature of the experiment / Portion of the intestine	Measurement	Initial control	Final control	Time after operation		
				10 days	1 month	2 months
Jejunum	Length	250	250	340	350	300
Ileum		190	200	320	300	280
Jejunum	Width	90	90	100	80	100
Ileum		90	90	90	100	100



The mucous membrane of the intestine in the same rat.
a) From the resected portion at the moment of operation; b) From the residual portion of the intestine 1 month after operation. Hypertrophy of the villi can be seen. Stained with hematoxylin-eosin. Magnification: ocular 5 ×, objective 12×.

TABLE 5

Mean Number of Cells per Unit Length of the Epithelial Layer of the Middle Portion of the Villi Before and After Resection of the Intestine

Nature of the experiment / Portion of the intestine	Initial control	Final control	Time after operation		
			10 days	1 month	2 months
Jejunum	34,3	33,6	31,2	31,5	31,2
Ileum	32,5	33,0	32,7	32,1	30,5

duodenum, the middle portion of the jejunum, the middle portion and end of the ileum in the fixed intestine.

EXPERIMENTAL RESULTS

The mean values of the length and area of the intestinal mucous membrane given in Table 1 show that in control (unoperated) rats the length of the intestine was increased by 27 cm in 2 months, whereas its diameter and the circumference of the mucous membrane was hardly changed. The degree of lengthening was 26%. The area of the mucous membrane of the intestine, relating to the crypt-bearing layer, was increased during this period by 23 cm^2.

After resection the length of the residual portion of intestine altered a little. The degree of its increase was 29%, i.e. the same as in the control rats. The area of the mucous membrane of the residual portion of the intestine almost reached that of the mucous membrane of the control rats only 10 days after operation, and remained for 2 months at roughly the same size as in the control rats of the same age. This was mainly due to an increase in the circumference of the mucosa of the residual portion of intestine: both jejunum and ileum. As may be seen from Table 1, after resection the diameter of the intestine also increased considerably. Many authors [4, 6, 8, 11] have pointed out an increase in the diameter of the residual part of the intestine when describing the morphological changes after resection. We did not, however, confirm the findings of Monari [8] that there was a shortening of the residual intestine after resection.

Counting of the number of villi (Table 2) in total preparations of the fixed intestine showed that their number in a certain area of the duodenum and the terminal ileum at all stages after operation differed only slightly from their number in the final control rats.

There was a small increase in the number of villi per unit area in the middle portion of the jejunum and ileum. This took place due to the fact that there was either an increase in the number of foliaceous villi or an increase in the degree of their extensibility in a transverse direction. The same change was observed in the final control rats compared with the initial control.

The area of the intestinal mucosa had increased by 28 cm^2 10 days after the operation (this group was compared with the final control rats at the moment of operation and after being killed), and by 51 cm^2 2 months afterwards. At the same time the number of villi per unit area was only very slightly altered. From this it could be assumed that after resection of the intestine the total number of villi was increased.

Further analysis of the experimental results showed that the structural reorganization took the form not only of an increase in the area of the intestinal mucosa and the number of villi, but also of changes in the thickness of the crypt-bearing layer and in the length of the villi. In Table 3 are given the mean values of the thickness of the crypt-bearing layer of the mucous membrane, and in Table 4 – the length and width of the villi.

As is evident from Table 3, the thickness of the crypt-bearing layer of the mucous membrane in the initial and final control rats was roughly equal. By the 10th day after operation it had increased almost twofold. Two months after operation the thickness of the crypt-bearing layer in the jejunum diminished, although its value was higher than that of the control animals. Thickening of the crypt-bearing layer was noted in both the jejunum and ileum.

It can be seen from Table 4 that the dimensions of the villi in the initial and final control rats were the same; the length of the villi in the jejunum was 25% greater than in the ileum. After resection of the intestine the length of the villi increased considerably, whereas their width did not perceptibly alter. The villi of different portions of the intestine were close to each other in size.

By way of illustration we show microphotographs of sections through the intestine of one of the rats (see Figure).

Counting the number of cells per uniform area of the epithelial layer of the villi showed that their number was unchanged after resection of the intestine, i.e. the hypertrophy of the villi and the increase in their number takes place at the expense of hyperplasia of the cells (Table 5).

In this investigation we were unable to confirm Monari's findings of an increase in the number of lymphatic follicles after resection of the intestine. The number of lymphatic follicles in the experimental rats was less than in the controls. If in the experimental groups, the number of lymphatic follicles in the resected portion of

the intestine was added to the number of lymphatic follicles in the residual area, then the values observed in the experimental and control rats differed little from each other (variation between 16 and 19).

It has been demonstrated by much experimental and clinical data that the removal of considerable areas of the small intestine is possible without harmful consequences for the body. This shows that "processes of regulation" (M. A. Vorontsova and L. D. Liozner [2]) take place in the intestine after resection, dispelling the consequences of the operation.

Until the present time the processes taking place in the intestine after resection have been regarded by all authors, including Monari [8] and Bornstein [6], as compensatory. The abundant experimental evidence of regeneration of the internal organs, together with modern theoretical considerations, compel us to re-examine this problem. It is now accepted that phenomena of regeneration may be displayed not only on the wounded surface, but in the residual portion of an organ. Under these circumstances we are concerned not only with hypertrophy, but also with the formation of new structural units within the organ. L. D. Liozner [3] suggested the name endomorphosis for this type of regenerative process, which is particularly observed in regenerative hypertrophy. These new ideas and the experimental findings of ours, in rats, suggest that the proliferative processes developing in the residual portion of the intestine after resection may be reparative processes of the endormorphosis type, taking the form of hypertrophy of the villi and an increase in their number. The investigation of the processes of repair in the intestine are even more interesting because after resection of a portion of the intestine there is no wounded surface.

SUMMARY

One third to one half of the medial portion of the small intestine was resected in white rats. The length of the remaining intestinal portion remained unchanged, but its diameter increased. The area of the intestinal mucous membrane of the operated rats in 10 days after the resection reached that of control rats. The structural reconstruction after the resection consisted in the hypertrophy of the villi and their increased number. These changes occurred in the jejunum and ileum and were retained for 2 months after the operation.

LITERATURE CITED

[1] P. P. Bryukhanov, Studies of the Digestive Processes in Various Defects of the Intestinal Region. Dissertation, St. Petersburg, 1913.*

[2] M. A. Vorontsova and L. D. Liozner, Physiological Regeneration, Moscow, 1955.*

[3] L. D. Liozner, Proceedings of a Conference on Problems of Regeneration and Cell Proliferation, pp. 39-41, Moscow, 1958.*

[4] B. D. Stasov, Studies of Compensatory Phenomena after Resection of the Intestine. Dissertation, St. Petersburg, 1913.*

[5] A. Albu, Berlin klin. Wschr. 50, 1248-1252 (1901).

[6] F. Bornstein, Arch. f. Path. Anat. 291, 921-930 (1933).

[7] G. Diliberti-Herbin, Zentr. f. Chirurgie. 4, 105-106 (1904).

[8] U. Monari, Beitr. z. klin. Chir. 16, 479-485 (1896).

[9] I. Nagano, Beitr. z. klin. Chir. 38, 2, 431-444 (1903).

[10] C. Schlatter, Beitr. z. klin. Chir. 49, 1-25 (1906).

[11] I. Soyesima, Deutsche. Zeit. f. Chir. 112, 4-6, 425-466 (1911).

[12] R. Trzebicky, Arch. f. klin. Chir. 48, 1, 54-92 (1894).

* In Russian.

THE REGENERATION OF SKELETAL MUSCLE IN MAMMALS UNDER NOVOCAIN BLOCK

N. I. Grebenskaya

From the Department of General Biology (Head - Prof. G. M Litver)
of the First Leningrad Medical Institute (Director - Docent A. I Ivanov)

(Received May 16, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

The method of novocain block, developed by A. V. Vishnevskii, has been widely sued in medical practice in the treatment of a variety of diseases. It has been shown that by its use the intensity of the inflammatory reaction is considerably reduced, and that inflammation in the stage of a serous effusion may even undergo regression [1, 2].

A differing action of novocain on the inflammatory reaction, depending on the place of its injection, was observed by K A Meshcherskaya, N. Melent'eva and G. Obogrelova [3]. These workers also found a weakening of the inflammatory reaction in the spleen after subcutaneous injection of novocain, and conversely, intensification of the inflammation after intraperitoneal injection.

In view of the statements of certain authors that the failure of regeneration of striped muscle after mechanical trauma is dependent on a well-developed inflammatory reaction and subsequent connective tissue reaction, we carried out histological investigations in order to study the effect of novocain block on the process of repair of skeletal muscle.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature white mice of both sexes, weighing 20-22 g. The calf muscles were used in the investigation. By means of ophthalmic scissors a linear incision was made in the muscles in their middle third, through half their thickness. Aseptic precautions were observed during the operations. Altogether 134 white mice were subjected to operation.

The experiments consisted of 3 series:

Series I – a study of the course of regeneration in control animals.

Series II– observations on regeneration during paranephric block (this series was performed on males only): immediately after operation an injection of 0.2 cm^3 of a 0.25% solution of novocain was given into the paranephric cellular tissue, alternately on the right and left sides (the injection was subsequently repeated after every second day).

In setting up this series we took into consideration the findings of several workers, especially L. N. Vlasova [4], that paranephric block reduces the intensity of an inflammatory process.

Series III – examination of the special features of regeneration after the injection into the lumbar division of the sympathetic trunk of 0.2 cm^3 of a 0.25% solution of novocain or 0.2 cm^3 of physiological saline. Injections were given immediately after operation, and then either every day (4-5 injections in all) or every second day (4 injections).

The role of the sympathetic system in the processes of regeneration was studied by A. Yu. Sozon-Yaroshevich [5]. This author showed that extirpation or division of the sympathetic trunk supplying the limbs had a favorable effect on the processes of healing; stimulation of various divisions of the sympathetic system, and in particular the ganglia, had the opposite effect.

The muscles were fixed in Zenker-formol. Paraffin wax sections, 6-8 μ in thickness, were stained with azure II-eosin, Heidenhain's iron-hematoxylin, Mallory's mixture, and survey films by Carazzi's hematoxylin.

The description of the course of regeneration in the control animals will be utilized later only for purposes of comparison, in view of the large number of references to it in the work of other investigators.

EXPERIMENTAL RESULTS

The difference between the controls (series I) and series II was clearly visible on only the second day after operation, and concerned the phenomena of immigration of blood cells into the focus of injury, and of phagocytic activity.

Whereas in the control mice on the 2nd day a large number of immigrating cells could be detected, both between and within the destroyed muscle fibers, and many phagocytes were present in necrotic masses of debris, in the series II animals at the same time small numbers of cells were seen between the damaged fibers, but the necrotic masses of debris contained hardly any phagocytes

In this way a delayed resorption of the necrotic masses of debris took place, and hence on the 4th day and sometimes at later periods a large number of unabsorbed remnants of damaged muscle fibers remained.

Proliferation of connective tissue cells lagged behind the control animals: the collagen fibers that formed were thin; the development of collagen bundles was delayed, the delay being greater the more injections were given. The connective tissue intermuscular septa remained poor in cells throughout the whole period of regeneration.

Our findings were in agreement with the observations of A. S. Ershtein [6], who studied the effect of novocain block on the processes of sensitization and found weakening of the Schwartzman phenomenon, and in particular a reduction in the infiltration by cells (histiocytes).

Another difference between series II and the control animals was the greater regularity of growth of the muscle fibers and also the small diameter of the regenerating muscle formations, giving the impression of dystrophy. This may possibly be the consequence of failure of development of the cells of the interstitial connective tissue which is known to carry out a trophic function.

In the series II animals, compared with the controls, there was some degree of retardation of differentiation of muscle cells. However, by the 25th day, all these differences had become to a large extent obliterated, apart from the fact that the regenerating muscle fibers were arranged in a more parallel fashion.

In relation to the mechanism of action of a paranephric novocain block, there are findings, in particular those of T. S Miryushchenko [7], that the block brings about phasic changes in the nervous processes in the central nervous system in which inhibition predominates.

Before turning to the description of the series III experiments, we must emphasize that the differences between the experiments in which injections of novocain were given and those in which physiological saline was injected were not found, and the variations from the controls were the same in both cases. This is perfectly in order, since according to A. V. Vishnevskii [1] the essence of the novocain block is weak stimulation of corresponding areas of the nervous system, a result of which can be achieved by the use of other substances, as was pointed out by the originator of the novocain block method himself. The findings of A. M. Vikhert [8], who studied the course of the Arthus phenomenon during local anesthesia, are also in accordance with this view.

In a series of experiments, A. M. Vikhert used physiological saline instead of novocain and also observed some degree of acceleration of the development of the Arthus phenomenon. Further, despite the fact that the injections were given on the left, there were corresponding changes, but expressed to a lesser degree, in the right calf muscle, which must be explained by the presence of connections between the left and right sympathetic trunks, which were demonstrated in particular by G. A. Orlov and N. L. Batygina [9].

Differences between the control and series III animals were apparent on the first day after operation: on the 2nd day, in the mice of series III a larger number of phagocytes than in the controls could be detected among the damaged muscle fibers. It must be pointed out that besides necrotic masses of debris thickly strewn with phagocytes, there were also masses in which no leucocytes at all were present.

The connective tissue activity in individual cases in series III was higher than in the controls on the 2nd day, as shown by the formation of bands of fibroblasts — the result of the increased mitotic activity of these cells. The fact that the immigration of cells into the damaged muscle fibers continued intensively on the 3rd day also was remarkable, because on the 4th day sarcolemma tubes, densely packed with cells, were seen, with necrotic masses of debris included within them, whereas in the control animals the process of resorption was almost completed. It might appear strange that in spite of the increased phagocytic activity observed on the second day in series III, the process of resorption lagged behind the controls. This was due to the increased fibroblastic activity which exerted an inhibitory effect on phagocytosis and autolysis, and the preservation of necrotic debris for a long time in the region of the wound itself prolongs the process of immigration of blood cells into the focus. Also of importance was the accumulation in the region of the injury of the enzyme macrocytase, possessing a proteolytic activity, as a result of the large number of macrophages, a feature first pointed out by I. I. Mechnikov and later confirmed in special research by V. Z. Gorkin and M. N. Kondrashova [10]. The appearance of new areas of necrosis was due to the action of this enzyme.

The subsequent course of restoration of the muscle depended on the character of the injections. In cases where the injections into the sympathetic trunk were given daily, growth of the corresponding muscle fibers took place more energetically than in the experiments in which injections were given at intervals of 2 days. The reactions on the part of the connective tissue in the latter case were more severe, which led subsequently to the presence of a large number of connective tissue cells in the intermuscular spaces.

M. P. Galashnikova [11], who studied the vascular and temperature reactions in response to stimulation of the sympathetic nerve, pointed out that besides acting on the vessel wall, the sympathetic nerve also has a direct action on the biochemical processes in the tissues. This effect is usually inhibitory in character, but in response to repeated stimulation this author observed stimulation of tissue metabolism. This stimulation of tissue metabolism also led to the more energetic growth of the muscle formations, when injections were given daily into the sympathetic trunk.

An essential difference from the control series was the retention of necrotic masses of debris in the regenerating muscle throughout the entire period of regeneration, mainly when the injections were given at intervals of 2 days. Part of the necrotic masses of debris were remains of unresorbed areas of damaged muscle fibers, other parts were newly formed areas of necrosis.

In some cases the necrotic masses of debris filled the greater part of the regenerating tissue, and they were found up to the 25th day. In such cases, there was a particularly intensive reaction on the part of the connective tissue, as shown by an increase in the saturation of the connective tissue interspaces and the marginal areas of the necrotic masses with cells.

Experiments carried out on females revealed differences in the structure of the regenerating muscle between the males and females, both experimental and control. In the control females on the 6th, 8th and 15th days after operation, many necrotic masses of debris were detected. The corresponding event in the males took place after the fourth injection (at intervals of 2 days) of novocain and of physiological saline into the sympathetic trunk.

After injection of novocain into the sympathetic trunk of females, as a rule necrotic masses of debris were not present in the regenerating muscle, and less reactivity was observed on the part of the connective tissue.

DISCUSSION

Hence the paranephric novocain block brought about a fall in the intensity of the inflammatory reaction, and later, a diminution of the connective tissue reaction. In consequence of this, the growing muscle fibers took on a more parallel course although the fibers themselves were somewhat dystrophic in character.

During novocain block of the sympathetic trunk, on the other hand, a considerable increase was observed in the intensity of the inflammatory reaction. The reparative processes depended on the character of the

injections: in response to daily injections of novocain, signs of regeneration of the muscle formations themselves were predominant, but when the injections were given at intervals of 2 days, there was considerable development of connective tissue, rich in cells.

SUMMARY

The regenerative process of the skeletal muscles (m. gastrocnemius) was studied in experiments on white mice in conditions of novocain block. It was shown by the histological examination that the course of repair depends on the site of novocain injection. Paranephral novocain block caused a decreased intensity of inflammation and reduction of the reactivity of connective tissue. A parallelism of muscular fibers and their slight dystrophy was noted in these experiments.

A significant increase in the intensity of the inflammatory reaction was noted in novocain block of the sympathetic trunk (administration of 0.2 cc of 0.25% novocain solution). The regenerative process varied depending on the character of the novocain injection.

Thus, in daily administration of novocain, the prevalence of regeneration of the muscle formations proper was observed, while in injections with 2-day intervals a pre-elective development of connective tissue rich in cells was noted. The structure of the regenerate was different in the males and in the females.

LITERATURE CITED

[1] A. V. Vishnevskii and A. A. Vishnevskii, Novocain Block and Oil-Balsam Antisepsis as a Special Form of Pathogenetic Therapy, Moscow, 1952.*

[2] A. V. Vishnevskii, Arkh. Biol. Nauk SSSR. 61, 1 (1941).

[3] K. A. Meshcherskaya, N. Melent'eva and G. Obogrelova, Farmakol. i Toksikol, 16, No. 4 (1953).

[4] L. N. Vlasova, Research on the Use of Radioactive Isotopes, 1953.*

[5] A. Yu. Sozon-Yaroshevich, Transactions of the All-Ukraine Psychoneurological Academy. "Problems of trophic innervation." 3, 1935.*

[6] A. S. Ershtein, Problems of Clinical and Experimental Surgery, 2, 1953.*

[7] T. S. Miryushchenko, Khirurgiya, No. 2, 35-39 (1955).

[8] A. M. Vikhert, Arkh. Patol. 17, 3, 8-14 (1955).

[9] G. A. Orlov and N. I. Vatygina, Voprosy Neirokhir. 3, (1951).

[10] V. A. Gorkin and M. N. Kondrashova, Biokhimiya 18, 3 (1953).

[11] M P Galashnikova, Author's summary of Candidate's dissertation, Moscow, 1953.*

*In Russian.

THE EFFECT OF A FALL OF THE TEMPERATURE ON REGENERATION IN THE AMPHIBIA AND ON THE RESISTANCE OF THE REGENERATING TISSUE UNDER THESE CONDITIONS TO THE CYTOTAXIC ACTION OF NOVEMBICHIN AND TEM

E. Ch. Pukhal'skaya

From the Laboratory of Chemotherapy (Head - Corresponding Member AMN SSSR Prof L. F. Larionov) of the Institute of Experimental Pathology and Therapy of Cancer (Director - Corresponding Member AMN SSSR N. N. Blokhin) of the AMN SSSR, Moscow

(Received May 29, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

A marked fall in the activity of metabolic processes in tissues in a state of hypothermia is known to occur, so that this state has been defined as one of rest from the energetic and functional activities of the cell or animal.

A characteristic feature of poikilothermic animals is their adaptation to a wide range of temperatures, although this refers only to their basal metabolism. So far as processes of growth, proliferation and regeneration are concerned, demanding a more intensive metabolism and, in particular, an increase in processes of synthesis, these are always adapted to a narrow range of specific positive temparatures [9].

On the basis of work on the production of synchronous division in cultures of protozoa [13] and bacteria [11], and also in tissue cultures [7], using the method of so-called temperature shock with subsequent return to the optimum temperature, it follows that before cell-division can take place, certain processes must be carried out which are sensitive to changes of temperature and which are artificially inhibited by such changes until such time as the optimal temperature is restored.

The object of the present investigation was to ascertain if it was possible to inhibit growth in regenerating organs of amphibia by means of slight hypothermia, and to study the cutostatic effect of compounds such as colchicine [10], novembichin and TEM [2] while the energetic and functional activities of the animals were thus in a slightly depressed state.

EXPERIMENTAL METHOD

The objects investigated were the regenerating tails of tadpoles (Rana temporaria and Rana ridibunda) of stages II and IIIa (Blacher). The experiments were performed during June - July, 1957.

In order to determine the mean rate of growth of the regenerating tail at a particular temperature, one fifth the length of the tail was amputated in 25 tadpoles, after which the length of the regenerated portion was measured every 4 days by means of an ocular micrometer under a loupe (ocular 10, objective 1.75). The experiment continued for 14 days. The arithmetic means of each of the three measurements were calculated and used to construct a curve of growth of the regenerating tail.

Each experimental study of the effect of the cytostatic drugs was also carried out on 25 tadpoles, from which the tip of the tail was amputated immediately after a single injection of the drug. The control series (amputation of the tail, but no drug administered) consisted of 25 tadpoles. The regenerating tails were measured just as in the previous experiments

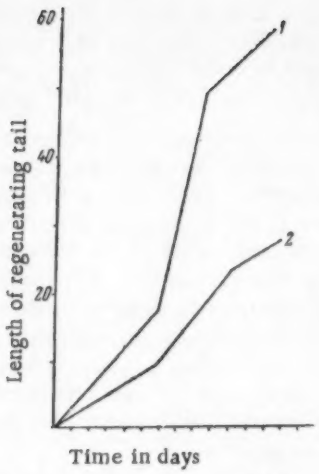


Fig. 1. Rate of growth of the regenerating tails.
1) At optimal temperature;
2) at a lowered temperature.
Abscissae – time in days from the moment of amputation.
Ordinates – length of regenerating tail in divisions of the ocular micrometer (one division equivalent to 0.087 mm).

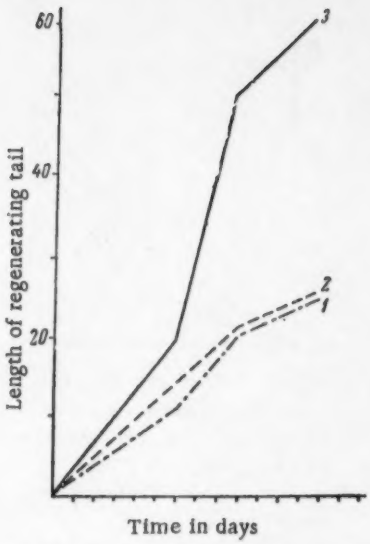


Fig. 2. Rate of growth of regenerating tail at optimal temperature with the action of novembichin (1), TEM (2) and control (3).

The method of keeping the tadpoles for predetermined periods of time in solutions of the test substances is very unsuitable, for it does not allow the dose of the drug ingested by the tadpole to be measured and also many of the drugs are rapidly hydrolyzed in aqueous solution.

Intraperitoneal injection of the tadpoles was thought to be impossible in view of their very high intraperitoneal pressure and the feeble development of the musculature of their abdominal wall.

We used a simple method of injection of the solutions into the peritoneal cavity of the tadpole. The injection needle must first be passed through the musculature of the tail a short distance from its base, and its tip is then pushed through into the peritoneal cavity. The rapid contraction of the muscles of the tail after withdrawal of the needle prevents escape of the injected fluid back along the puncture wound. This method of injection is especially convenient in the larger tadpole of Rana ridibunda. Doses are calculated per kg body weight.

Since tadpoles of the same species and stage, taken from the same tank, are sufficiently homogeneous experimental material, as a preliminary measure we weighed 5-6 specimens taken at random and determined the mean weight for this particular stage from which we calculated the dose. Altogether 10 experiments were carried out. The preparations were injected in physiological saline (0.65% solution of sodium chloride).

EXPERIMENTAL RESULTS

A mean daily temperature of the external environment of 18-20° is the optimum at which rapid growth of the regenerating tail was observed (Fig. 1, 1). When the water temperature was lowered by 2-4°, i.e. at an average daily temperature of 14-16°, an obvious fall in the rate of growth of the regenerating tail was observed (Fig. 1, 2).

Subsequently, the regenerating tails growing intensively at optimal temperature will be referred to as type A, and those whose retarded development was adapted to lowered temperature conditions, as type B. If the mean length of the rapidly growing type A regenerating tail on the 14th day was taken as 100%, then the difference between it and the length of the type B tail, expressed as a percentage of the length of type A, was 60%.

It could thus be concluded that a fall in the average optimal temperature pf 2-4° inhibits the regeneration of the tail of the tadpole by 60%.

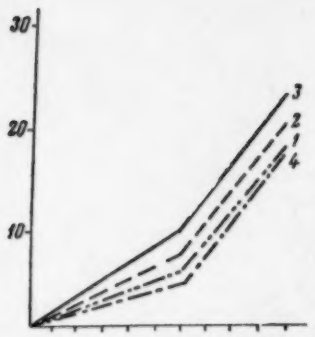


Fig 3. Rate of growth of the regenerating tails at a lowered temperature and under the action of novembichin (1), TEM (2), control (3) and of a double dose of TEM (4) (16 mg/kg).

The mitotic coefficient for the type A regenerating tail, estimated for 10,000 cells of the epidermis of the regenerating tail and of the adjoining 5 mm of the residual organ, was 2.5% on the 9th day after amputation. The mitotic coefficient for the type B regenerating tail under the same conditions of counting was 0.8%.

Preliminary experiments to determine the toxicity of the tested compounds to tadpoles showed that the LD 100% (the dose lethal to 100% of animals) of novembichin and TEM for tadpoles of stage II and IIIa per kg of body weight was 10-12 times as high as the LD 100% for rats. A single dose of TEM of 8 mg/kg, and of novembichin of 10 mg/kg was well tolerated by the tadpoles, i.e. if the LD 100% of these drugs for rats, which has been well studied, was taken as n, the single dose tolerated by tadpoles was approximately 4n. Hence the cytostatic action of these two compounds could be compared if the dose used was equivalent to 4n.

In respect of colchicine, however, the LD 100% for tadpoles only exceeds the LD 100% for rats per kg body weight by $1^1/_2$ times. For this reason colchicine was given in a dose of 0.6 mg/kg, i.e. a dose equal to n. The action of novembichin and TEM in equivalent doses on the type A regenerating tail took the form of considerable inhibition of growth of the tail (Fig 2, 1 and 2).

If the degree of inhibition of growth is represented as the difference between the lengths of the regenerating tail in the experimental and control groups, expressed as percentages of the control values, the inhibition of type A regeneration by novembichin and TEM would be about 60% (57 and 54%). The inhibitory action of colchicine on type A regeneration was significantly weaker – 23% in all.

The type B process of regeneration was more resistant to the action of novembichin and TEM (Fig. 3, 1, 2), for hardly any inhibition of growth of the regenerating tail was observed (13 and 17%). Doubling of the dose of novembichin (20 mg/kg) caused practically no increase in the effect. Doubling the dose of TEM (16 mg/kg) led to some intensification of inhibition of growth of the regenerating tail (up to 23%) (Fig. 3, 4). On the other hand, colchicine had an equally inhibitory effect on both type B and type A regeneration (27%). In a lethal dose (1 mg per kg) colchicine caused total inhibition of both type A and type B regeneration, and the animals died on the 7th-8th day.

Certain phases of synthesis possibly exist in the life cycle of the cell which are particularly sensitive to some injurious agents, one of which may block these phases and produce a certain degree of resistance of the cells to the subsequent action of other injurious factors, i.e. may produce nonspecific resistance of the cell to several injurious factors. M. N. Meisel' and his co-workers [4], for example, established that yeast cells, exposed to the toxic action of berberine, thioflavine or ethyl alcohol, became resistant to the action of ionizing radiation.

On the other hand, it has been found in plants and cultures of protozoa [1, 5] that if cells acquire resistance to a superoptimal temperature they will also be resistant to a lowered temperature and to the action of certain noxious chemical agents In such cases signs of weakening of the processes of synthesis are observed, namely: retarded growth [6] and lowering of enzyme activity [12] of tumors resistant to the chloroethylamines, slow growth of regenerating tissues resistant to TEM and novembichin (in our experiments), depression of photosynthesis in the leaves of plants resistant to a raised temperature [3] and so on.

On the basis of the information in the literature on this subject [1, 8, 12] it can be assumed that the fall in metabolism to a certain extent, mainly at the expense of depression of oxidative processes, is an important condition for the acquisition by the cells of resistance to the action of certain injurious factors.

The resistance of type B regeneration to the action of novembichin and TEM in our experiments is presumably also due to a fall in the metabolic activity of the tissues concerned.

SUMMARY

The average velocity of growth of the tail regenerate in Rana temporaria and R. ridibunda tadpoles of the II and III stage (according to Blacher) was studied at various temperatures. It was demonstrated that the average 24-hours temperature of 18-20° C is the optimal for the growth of the regenerate. By decreasing the water temperature by 2-4° C the velocity of the regenerate's growth is retarded by 60% (in comparison with the growth at the optimal temperature). The author studied the effect of colchicin, novembichin and 2, 4, 6 triethyleneimio 1, 3, 5-triazine (TEM)on the regeneration of the tail at optimal temperature (type A), as well as in its regeneration at decreased temperatures (type B). The technique of administration of these preparations into the tadpole body cavity was developed and LD 100% determined. The process of regeneration in type B revealed the resistance to the effect of novembichin and TEM.

LITERATURE CITED

[1] V. Ya. Aleksandrov and N. L. Fel'dman, Bot. Zhur. 43, 2 194-213 (1958).

[2] L. F. Larionov, Patol. Fiziol. i.Eksptl. Terap. 1, 3 14-21 (1957).

[3] M. I. Lyutova, Bot. Zhur. 43, 2 283-287 (1958).

[4] M. N Meisel', N. A. Pomoshchnikova and T. S. Sokolova, Doklady Akad. Nauk SSSR, 117, No. 1, 142-145 (1957).*

[5] Yu. I. Polyanskii (assisted by A. F Orlova), Zool. Zhur. 36, 11 1630-1647 (1957).

[6] L. F Sharlikova, Voprosy Onkol. 1, 6 74-79 (1955).

[7] S. Chevremont-Comhaire and M. Chevremont, Compt. rend. Soc. Bio. 150, 1046-1049 (1956).

[8] J. Christophersen and H. Precht, Biol. Zbl. 72, 104-119 (1953).

[9] A. Kanitz, Temperatur und Lebensvorgange, Berlin 1915.

[10] M. Luscher, Rev. suisse, zool. 53, 683-734 (1946).

[11] Y. X. Maruyama and T. Yanagita, J Bact. 71, 542-546 (1956).

[12] Y. Miura, H. Katayama and M. Goto, Abstr. Sympos. chemother cancer Tokyo, 1957.

[13] O. Sherbaum and E. Zeuthen, Exper. cell. research 6, 221-227 (1954).

* See English translation.

THE REGENERATIVE POWER OF THE HYPERTROPHIED LUNG OF THE CRESTED TRITON

L. K. Romanova

From the Laboratory of Growth and Development (Head - Prof. L. D. Liozner) of the Institute of Experimental Biology (Director - Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received August 7, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

A result of the successful development of the surgery of the lungs during the last 50 years was the wide use of various operative procedures on these vitally important organs. In a number of cases on clinical grounds it is necessary to operate on a lung which has already suffered damage, or on a lung in which compensatory hypertrophy has taken place because of absence of the other organ of the pair. Since we were unable to find in the accessible literature any mention of the subject of the regenerative power of the hypertrophied lung, we deemed it of interest to find out whether the hypertrophied lung was capable of regeneration. Moreover the hypertrophied lung might be expected to show changes after injury representing the combined effect of compensatory hypertrophy and regeneration. For this reason our investigation had also to include the delineation of these two processes and the study of their special individual features. However, the investigation of this problem in mammals is attended by great difficulties both of performance of the experiments and of analysis of the results. This led us to seek a new experimental model on which to study the laws of regeneration of hypertrophied lungs, using for this purpose animals readily accessible for experimental procedures and permitting a comprehensive study of the processes of regeneration.

The caudate amphibia satisfy these requirements.

EXPERIMENTAL METHOD

Two main series of experiments were carried out on the crested triton (Triturus cristatus), of both sexes and weighing 4500-7000 mg. In the first series of experiments compensatory hypertrophy was first induced in the right lung of the animals by removal of the left lung, and 2 months after this operation one half of the hypertrophied lung was resected (operations performed on 23 tritons of which 17 survived).

So that the character of the restorative process taking place in the injured, hypertrophied lung could be compared with the process of regeneration of normal lung, we carried out a second series of experiments in which the left lung was removed completely and at the same time one half of the right lung was resected (operations performed on 49 tritons of which 27 survived).

As a main control we used tritons on which no operation was performed, and which were kept under the same conditions as the animals undergoing operation.

Furthermore, in order to discover whether there were any differences in the course of the continuing compensatory hypertrophy, on the one hand, and the regeneration of the injured, hypertrophied lung on the other, a supplementary control was set up. Compensatory hypertrophy ran its course in the right lung of the animals of this control group for 4 months after removal of the left lung (operation performed on 30 tritons of which 20 survived).

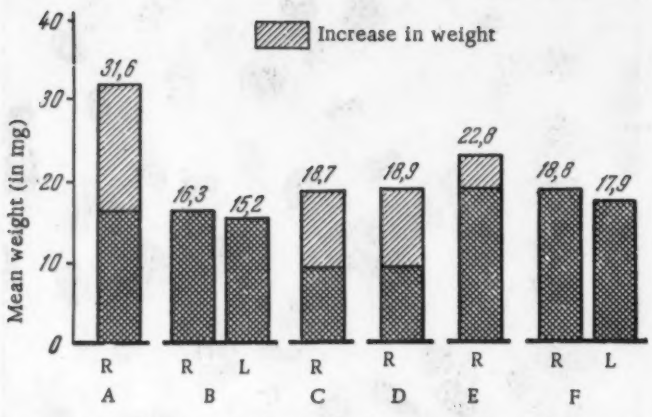


Fig. 1. Mean weight of the lungs of tritons 2 months after operation R) Right lung; L) left lung; A) removal of the left lung B) control of A; G) series I (removal of half the right lung 2 months after left-sided pneumonectomy) D) series II (simultaneous total removal of the left and half the right lung); E) removal of the left lung Period of observation four months. F) controls of series I and II and of E.

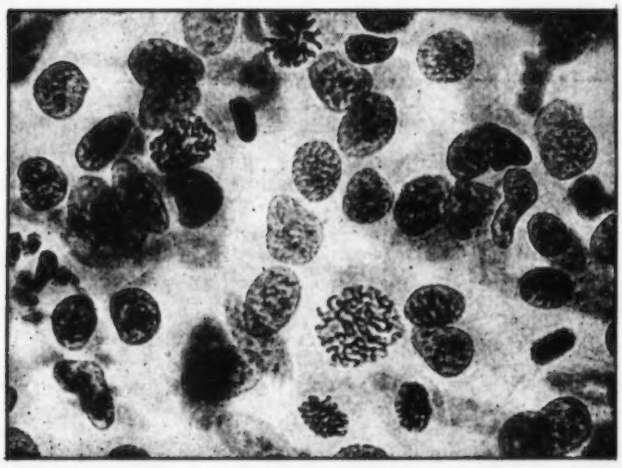


Fig. 2. Mitoses in the cells of the pulmonary epithelium of the regenerating lungs. Total preparation. Magnification: ocular 5×, objective 100 ×.

When the entire lung was removed, the resection was made at the point of its separation from the laryngotracheal division; when half the lung was removed, this was done at the level of the commissure joining the lung to the sex gland.

Two months after operation (in the first series 2 months after the second operation) the tritons were killed.

The lungs were fixed in a state of inflation in Bouin's fluid. Total lung preparations were stained with hematoxylin-eosin and with hemotoxylin and counterstained with picrofuchsin, and mounted in balsam so that the internal surface of the lung, lined with pulmonary epithelium, was turned toward the cover glass. In the films (4-5 cases from each series and from the controls were taken) we measured the width of the lungs, counted the cells of the pulmonary epithelium in one field of vision and estimated the average number of mitoses per

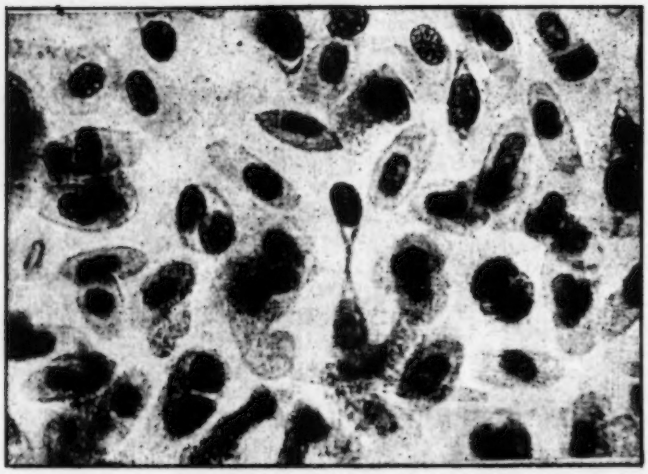


Fig. 3. Amitosis in a cell of the pulmonary epithelium of the regenerating lung Total preparation. Magnification: ocular 5x, objective 100x.

4000 cells. By means of drawings we determined the area of the nuclei of the cells of the pulmonary epithelium, which enabled us to judge their hypertrophy.

EXPERIMENTAL RESULTS

Regeneration of the lung after simultaneous removal of the left and half of the right lung.

In the animals of this series 2 months after operation the lung was shaped like a small sack, the distal end of which was rounded and dilated. In its color and blood content, there was little difference between the operated and unoperated lungs. At the place of union of the edges of the wound in the lung there was a small white scar, but in no case were outgrowths of lung tissue observed from the raw surface.

Besides these findings a considerable increase in the weight of the regenerating lung took place (Fig. 1). The increase in weight of half of the regenerating lung by comparison with half of the normal lung was 101% (18.9-9.4 mg). We observed changes in weight of the same order on comparing the relative weight of the operated and normal lungs. This increase in weight was statistically significant.

The mean absolute and relative length of the resected lung was almost unchanged by comparison with the length of half the normal lung.

The increase in the size of the residual portion of the lung took place largely on account of changes in its width, which increased by 18%. The regenerating lung differed in its histological structure from the undamaged lung of the triton: its walls were thicker than the walls of the normal lung, and its muscle cells were hypertrophied. Whereas in the normal lung the cells of the pulmonary epithelium were encountered in groups containing 2, 3 or at the most 4 nuclei, in the regenerating lung larger collections were found, containing 8-10 nuclei. Also, the number of these collections in the regenerating lung per field of vision was less than in the normal. The nuclei of the pulmonary epithelial cells were of different shapes and sizes, whereas the nuclei of the pulmonary epithelial cells in the normal lung were characteristically of average size and oval in shape.

The increase in the number of nuclei in the pulmonary epithelial cells and their polymorphism all indicate the presence of proliferative processes. It was found that the mitotic coefficient (MC) in the epithelium of the regenerating lung in the animals of the 2nd series was 250% higher than that in the epithelium of the lungs of the control tritons. This was suggestive of intensive hyperplasia of these cells.

The ratio between the phases of mitosis was the same as in the lungs of normal tritons. No special zones of mitotic activity could be identified, and only along the course of the pulmonary artery were dividing cells

TABLE 1

Changes in the Regenerating Hypertrophied Lung 2 Months after Operation

Series of experiments	Type of operation	Number of animals	Period of observation (in months)	Right lung						
				Length (in cm)	Width (in cm)	Mitotic coefficient (in %)	Area of nuclei (in relative units)	Increase in mitotic activity (in %)	Degree of hypertrophy of nucleus (in %)	Ratio of intensity of hyperplasia to hypertrophy
1	Removal of half of the hypertrophied lung	17	2	1.7	1.08	20.2	76	260	10	26.0
2	Simultaneous removal of the left lung and half of the right control	27	2	1.5	1.08	19.6	78	250	13	19.2
3	Total removal of the left lung	20	4	3.1	1.09	4.4	86	22% below normal	24	—
	Control	15	—	3.1	0.92	5.6	69	—	—	—

TABLE 2

Degree of Compensatory Hypertrophy in the Lung in Relation to the Time after Removal of the Opposite Lung

Type of operation	Period of observation (in months)	Right lung		
		Weight (in mg)	Increase in mitotic activity (in %)	Degree of hypertrophy of the nucleus (in %)
Removal of the left lung	2	31.6	422	43
	4	22.8	22% below normal	24

observed slightly more often than in the remaining lung tissue. Proliferation of the cells of the pulmonary epithelium was not confined to division of cells by mitosis: their proliferation by amitosis could also be observed (Fig. 2, 3).

Comparison of the area of the nuclei of the pulmonary epithelial cells in the lungs of the experimental and control animals showed that hypertrophy of the nuclei of the cells of the regenerating lung took place (Table 1).

In summing up, it must be pointed out that in the regenerating lung of the triton after removal of the opposite lung, the processes of proliferation of the pulmonary epithelial cells and of hypertrophy of their nuclei were intensive and prolonged.

The result of this was an increase in the weight and volume of the residual organ. It might be thought that two processes were taking place simultaneously in the residual organ: compensatory hypertrophy, since restoration took place in the absence of the other paired organ, and regeneration, since part of the lung had been removed.

It was, therefore, natural to consider that restoration of the traumatized organ in an experiment under these conditions was the result of these two processes taking place in harmony and in mutual association.

Also, we were inclined to think that the deciding factor in this case was the injury to the lung, the presence of which was responsible for the special features of the restorative process.

Regeneration of the lung took place along the lines of regenerative hypertrophy, like that which was shown in a series of investigations proving that restoration of the internal organs (lungs, kidneys, pancreas and salivary glands) of mammals takes place mainly by regenerative hypertrophy [1, 2, 3, 4, 5].

Regeneration of the hypertrophied lung. In the experiments of series I, after removal of half of the hypertrophied lung the same results in general were obtained as after simultaneous removal of the whole lung and the half.

The regenerating hypertrophied lung weighed on the average 18.7 mg. If it is considered that at the moment of resection this lung weighed on the average 31.6 mg, as shown by the appropriate data (Fig. 1), then the half of the regenerating hypertrophied lung increased in weight on the average by 2.9 mg (18%) in comparison with the part left behind after operation (18.7-15.8). Furthermore, the regenerating hypertrophied lung overtook in weight the lung of the control animals, which weighed 18.8 mg. In relation to the half of the control lung, the increase in weight of the regenerating lung averaged 98% (18.7-9.4 mg). Such a gain in weight, however, might be regarded as the result either of regeneration or of continuing compensatory hypertrophy. The latter hypothesis breaks down, since comparison of the results obtained in animals killed 2 and 4 months after removal of one lung suggested that the process of compensatory hypertrophy not only did not progress but actually gradually receded: the weight of the hypertrophied lung was reduced, and both the mitotic activity of the pulmonary epithelium and the degree of hypertrophy of the nuclei decreased. What is more, the mitotic activity of the pulmonary epithelium of the lung in which compensatory hypertrophy had lasted for 4 months was less than that of the pulmonary epithelium of the lungs of the control tritons (Table 2).

Thus during the same period of time (2 months) the regenerating hypertrophied lung increased in weight by 2.9 mg (18%) in relation to its original condition, whereas the lung in which compensatory hypertrophy continued not only did not gain in weight but actually weighed 8.8 mg (28%) less than the left lung, the compensatory hypertrophy in which had been going on for longer than 2 months. This indicated that the processes observed in the resected and in the undamaged hypertrophied lungs were dissimilar in their nature. Hence the increase in weight of the injured hypertrophied lung was the result of regeneration and not of compensatory hypertrophy.

It follows from the results described that the hypertrophied lung of the triton is capable of regeneration which takes the form of regenerative hypertrophy, and during this process the ratio between the indices of hypertrophy and hyperplasia of the cells of the pulmonary epithelium remains almost the same as during regeneration of the lung which has not previously undergone any form of operation but with the opposite lung removed.

Attention must be drawn to the possible appearance of regenerative powers in the hypertrophied lung in spite of the extinction of the process of compensatory hypertrophy in the lung.

The fundamental hypothesis may therefore be put forward that the stimulus which mobilizes the reserve powers of the body and which is capable of displaying the regenerative powers of the hypertrophied lung is the factor of trauma and the removal from the body of a definite amount of functioning lung tissue.

SUMMARY

Compensatory hypertrophy was induced in the right lung of tritons by removing the left lung. Two months after the operation half of the hypertrophied lung was resected. Comparison of the data obtained in this series of experiments and in the series where regeneration of the lung occurred after simultaneous removal of the whole lung on one side and one half on the other demonstrated that the hypertrophied lung of tritons is able to regenerate. Regeneration runs a course of regeneration hypertrophy. The relationship of the indices of hypertrophy and hyperplasia of the cells of the pulmonary epithelium is almost the same in both series of experiments.

LITERATURE CITED

[1] A. G. Babaeva, A Morphophysiological Analysis of Reparative Regeneration of the Parotid Salivary Glands in Guinea Pigs. Candidate's Dissertation, Moscow, 1956. *

[2] A. A. Birkun, Proceedings of a Conference on Regeneration and Cell Proliferation, 1958. *

[3] M. A. Vorontsova, Restoration of Lost Organs in Animals and Man, Moscow, 1953. *

[4] Yu. N. Kopaev, Proceedings of a Conference on Regeneration and Cell Proliferation, Moscow, 1958. *

[5] G. G. Samsonidze, A Morphophysiological Analysis of the Process of Regeneration of the Kidney after Injury, Tbilisi, 1958. *

* In Russian.

THE MITOTIC ACTIVITY OF THE CORNEA IN MICE AFTER INFLICTION OF A BURN AT DIFFERENT TIMES OF DAY

M. T. Gololobova

From the Laboratory of Histophysiology (Head - Candidate Bio. Sci. V. N. Dobrokhotov) of the Institute of Experimental Biology (Director - Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received June 20, 1958. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

There are reports in the literature that the infliction of an injury or burn leads to reactive changes in the surrounding tissues. It is also known that injury to one of a pair of symmetrical organs causes changes in the other. The infliction of a burn to one cornea in rats, for instance, leads to a persistent fall in the number of dividing cells in both the damaged and the intact (symmetrical) cornea [2, 3].

The existence of a diurnal periodicity of cell proliferation has recently been proved in the cornea of rats [1] and mice [4]. There are certain indications of possible changes in the diurnal periodicity of mitosis under the influence of trauma in the work of Jaffe [5], who studied regeneration of the liver in rats.

In the present investigation we studied the changes in the diurnal periodicity of cell division after the infliction of a burn, in the damaged and the symmetrical cornea, and also the changes in the diurnal periodicity in relation to the time of day at which the burn was inflicted.

EXPERIMENTAL METHOD

For the experiment we used 160 male mice, weighing from 20 to 25 g (110 mice in the experimental group and 50 in the control group). The diurnal rhythm of cell division in the cornea was studied after fixation of the material at intervals of 6 hours.

After decapitation of the animals the material was fixed in Zenker's fluid and stained with hematoxylin by Carazzi's method.

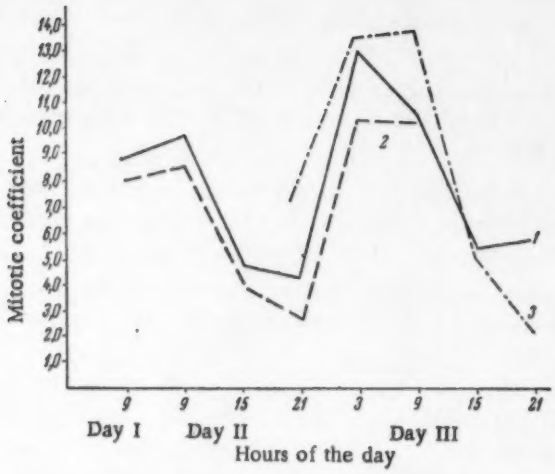
A burn was inflicted on the cornea of the right eye by means of a red-hot dissecting needle, in the first series of experiments this operation being carried out at 9 A. M. and in the second series at 9 P. M.

In order to count the dividing cells in the cornea, two lines were drawn at right angles to each other on the cover glass, and on each side of these lines all the fields of vision were examined in which mitoses were counted. On the average the number of fields of vision in one cornea was 145. In addition, in certain fields of vision the average number of cells was counted. The mitotic coefficient – i.e., the ratio of the number of dividing cells to the total number of cells – was calculated.

The whole of the numerical results were treated statistically by the Fisher-Student method.

EXPERIMENTAL RESULTS

Diurnal periodicity of cell proliferation in the normal cornea. The Figure shows graphically the changes in the mitotic coefficient in the course of 24 hours in the normal cornea and after burning. The results obtained



Changes in the mitotic coefficient in the cornea of a mouse in the course of 24 hours after infliction of a burn.
1) In the normal cornea; 2) after infliction of a burn in the morning (series I); 3) after infliction of a burn in the evening (series II).

indicate that in the epithelium of the cornea of normal mice there was a diurnal rhythm of cell proliferation. Starting at 9 A.M. the number of dividing cells gradually decreased, and reached a minimum at 9 P.M. The fall in the level of mitotic activity from 9 A.M. to 9 P. M. was statistically significant ($P = 0.001$).

At 3 A.M. a sharp rise in mitotic activity began, and this also was statistically significant ($P = 0.0001$).

At 9 A.M. on the following days a fall was observed in the number of dividing cells, but the difference in the intensity of cell division between 3 and 9 A.M. was not statistically significant.

These findings showed that there is a diurnal rhythm of mitotic activity in the cornea of normal mice: the curve was clearly unimodal in character.

Similar results have also been obtained by I. A. Utkin [4], but the diurnal rhythm of mitotic activity was examined by this worker in material fixed at only two times of day. Furthermore, we ourselves had previously shown the existence of the same sort of curve of diurnal rhythm of cell proliferation in the epithelium of the cornea, the epidermis and the small intestine of normal rats [1].

The level of mitotic activity in the cornea after burning. After infliction of a burn on the cornea the tempo of cell division there fell sharply after only 24 hours. The depression of mitotic activity 24 hours after infliction of the burn was statistically significant both in comparison with the normal cornea and in comparison with the opposite cornea to the one injured ($P = 0.0001$ and $P = 0.016$). Three days after burning the intensity of cell division in the injured cornea increased somewhat, but it remained at only half the level of that in the cornea of normal animals.

The fall in the value of the mitotic activity was statistically significant ($P = 0.017$ and $P = 0.027$).

Hence we could speak about a marked depression of the intensity of cell division in the cornea even three days after a burn had been inflicted on it.

Unfortunately it was not possible to study the changes in the cornea at other times during the experiment, since in the majority of cases the infliction of a severe burn led to a discharge of fluid from the eye and to shrinking of the cornea.

The diurnal rhythm of mitotic activity in the opposite cornea to the one injured, the burn being inflicted during the morning. The changes in the mitotic coefficient found in the 1st series of experiments showed that

the diurnal rhythm of cell division in the opposite cornea to the one damaged was preserved (see Figure). However, in material fixed at any time of day, the intensity of cell division here was rather below that in the cornea of the control animals.

The fall in the level of mitotic activity in the opposite cornea began, as it did in the normal cornea, at 9 A. M. The minimum number of divisions was observed at 9 P. M. The fall in the tempo of cell division at this time was statistically significant ($P = 0.0001$).

As in the cornea of normal mice, a sharp rise in mitotic activity was observed at 3 A.M. ($P = 0.0001$). At 9 A.M. on the third day after infliction of the burn, the number of dividing cells in the opposite cornea was approximately the same as that characteristic of the mitotic activity of normal mice.

Thus after infliction of a burn on the cornea of mice, during the morning hours, the diurnal rhythm of mitotic activity in the opposite cornea was preserved, although the intensity of cell division here was rather lower than in the cornea of the control mice.

The diurnal rhythm of mitotic activity in the opposite cornea to the one damaged, the burn being inflicted in the evening. In the 2nd series of experiments a burn was inflicted on the cornea in the evening (at 9 P.M.), i.e. at a time when under ordinary conditions the number of cell divisions in the cornea was minimal. Two days after infliction of the burn, the mitotic activity in the opposite cornea to the one injured was considerably higher than that in the cornea of the control animals and in the opposite cornea to the one injured when the burn was inflicted in the morning. The difference in the intensity of cell division in the corneas of the mice in the 1st and 2nd series of experiments (9 P.M.) was statistically significant ($P = 0.003$). At 3 A.M. a further increase in mitotic activity was observed ($P = 0.01$). However, in consequence of the considerable individual variations, the difference in level of the mitotic activity in the corneas of the mice in the 1st and 2nd series of this experiment at 3 A.M. was not statistically significant ($P = 0.05$). A sharp fall in the mitotic activity was then observed, continuing until 9 P.M. which indicated the possible compensation for the rise in mitotic activity.

Thus on the basis of the results of the 2nd series of experiments it could be concluded that after infliction of a burn to the cornea of mice during the evening hours, the diurnal rhythm of mitotic activity was preserved, although the intensity of cell division in this case was higher than when the burn was inflicted during the morning.

It is difficult at present to find the cause of this phenomenon. It can only be stated that the differences in the changes of mitotic activity after infliction of an injury to an animal during the morning or evening hours are not a chance happening, since our results are in agreement with those described in the paper by Jaffe [5].

It may thus be concluded from our results that the time of infliction of a burn has a bearing on the tempo of cell proliferation.

SUMMARY

The 24-hour rhythm of cellular division is preserved in symmetrical cornea by inflicting corneal burns in the morning. However, the average values of mitotic coefficients during the whole experiment appear to be lower than in the cornea of normal animals.

The infliction of burn in the evening has no effect on the character of the 24 hour rhythm. However the general level of mitotic activity is much higher. A stable depression of the tempo of cellular division was noted in the injured cornea.

LITERATURE CITED

[1] M. T. Gololobova, Byull. Éksptl. Biol. i Med. 9, 118-122 (1958).*

[2] L. N. Zhinkin and G. F. Korsakova, Doklady Akad. Nauk SSSR, 81, 5, 965-968 (1951).

[3] G. A. Kovaleva, Proceedings of a Conference on Regeneration and Cell Division, 25-27, Moscow, 1958.*

[4] I. A. Utkin, Voprosy Onkol. 1, 4, 3-11 (1955).

[5] J. Jaffe, J. Anat. Rec. 120, 4, 935-954 (1954).

*Original Russian pagination. See C. B. Translation.

**In Russian.

A COMPARATIVE STUDY OF THE ANTIGENIC PROPERTIES OF THE TISSUES OF DIFFERENT SPECIES OF ANIMALS AT VARIOUS STAGES OF DEVELOPMENT

COMMUNICATION III. A COMPARATIVE STUDY OF THE ANTIGENIC PROPERTIES OF THE HEART TISSUE OF THE FROG (RANA TEMPORARIA) AND THE TRITON (TRITURUS TAENIATUS)

R. F. Averkina

From the Laboratory of the Immunology of Embryogenesis
(Head - Candidate Med. Sci. O. E. Vyazov) of the Institue of
Experimental Biology (Director - Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Received April 14, 1958. Presented by Active Member AMN SSSR
N. N. Zhukov-Verezhnikov)

The problem of the changes in the antigenic properties of tissues of the amphibia in the course of their individual development is not yet solved. The results of research in this field are very contradictory. Some authors [2, 13] consider that the antigenic properties of tissues of the amphibia do not change in the process of development. These authors' findings are not, however, in agreement with the results of other investigations [3-7, 10-12].

In order to shed light on the problem of the changes in the antigenic properties of the tissues of amphibia in the process of ontogenesis, we decided to carry out a special investigation.

We also had the aim of ascertaining the degree of interspecies similarity of the antigenic properties of the tissues of larval and adult forms of Anura with the analogous tissues of adult members of Urodela.

The immunological study of the interspecies relationships, taking into consideration the various stages of development, appeared to us to be of interest, since research in this direction has been carried out only on adult forms [2, 8, 9]. The sole exception is the work of I. L. Krichevskii [7], devoted to the comparative study of the antigenic properties of the tissues of the tadpole and the adult frog and the antigenic properties of the tissues of the triton. We have carried out a similar sort of investigation [1], differing from Krichevskii's work by the fact that we used muscle tissue and not the animals in toto.

In the present investigation a comparative study of the antigenic properties of the tissues of the heart was made.

EXPERIMENTAL METHOD

In the experiments we used the heart tissues of the tadpole, the frog (Rana temporaria) and the triton (Triturus taeniatus). As antigens we used a saline extract of the heart tissues of tadpoles taken at various stages of development (0, I-III, IV according to L. Blacher), and also saline extracts of the heart tissues of the frog and triton. Immune rabbit sera were prepared against the saline extract of the heart tissues of the frog and triton. The method of preparation of the extract and of the immune sera was described previously [1]. Complement fixation tests were carried out with the sera obtained and with all the above-mentioned antigens.

TABLE 1

Results of the Complement Fixation Test by Antisera to the Heart of the Frog and Triton with Antigens from the Heart of the Frog, Tadpole and Triton

Sera	Dilution	Antigens from the heart				
		the frog	the tadpole, stages of development			The triton
			0	I–III	IV	
Against the frog's heart	1 : 20	++++	++++	++++	++++	++++
	1 : 40	++++	+++	+++	+++	+++
	1 : 80	++++	++	++	++	+
	1 : 160	+++	+	+	+	h
	1 : 320	+	h	h	h	h
	1 : 640	h	h	h	h	h
Against the triton's heart	1 : 20	+++	+++	+++	+++	++++
	1 : 40	++	+++	+++	+++	++++
	1 : 80	+	++	++	++	++++
	1 : 160	h	+	+	+	++++
	1 : 320	h	h	h	h	+++
	1 : 640	h	h	h	h	+
Normal	1 : 20	h	h	h	h	h
	1 : 40	h	h	h	h	h
	1 : 80	h	h	h	h	h
	1 : 160	h	h	h	h	h
	1 : 320	h	h	h	h	h
	1 : 640	h	h	h	h	h

Note: ++++ total absence of hemolysis; +++, ++, +, ± intermediate degrees of hemolysis; h – total hemolysis; a point indicates that no test was performed.

EXPERIMENTAL RESULTS

As may be seen from Table 1,* serum against the heart tissues of the frog reacted in the complement fixation test with homologous antigen in a dilution of 1:320, and with extract from the heart tissues of the tadpoles at the above-mentioned stages of development, in a dilution of 1:160. This serum gave the weakest reaction of all with the extract from the heart tissues of the triton (1:80). The serum against the heart tissues of the triton reacted with the homologous antigen in a dilution of 1:640, with the extract from the heart tissues of tadpoles taken in stages 0, I-III and IV of development in a dilution of 1:160, and with the extract from the heart tissues of the frog in a dilution of 1:80.

Comparison of the titers and the intensity of the reaction between the antiserum to the heart tissues of the frog and the extracts from the heart tissues of the frog and tadpoles showed that these heart tissues differed in their antigenic properties. The antisera to the heart tissues of the frog and triton reacted with the extract from the heart tissues of the tadpoles in various stages of development in the same dilutions and with the same intensity. Consequently the antigenic properties of the heart tissues of the tadpoles in all the three above-mentioned stages of development showed no difference in our experiments. Furthermore, comparison of the titers and intensity of the reaction of these two sera showed that the difference between the titer and intensity of their reaction with antigens of the tissues of the frog and triton was considerably greater than the difference between the titer and intensity of the reaction with antigens of the tissues of the tadpoles and triton. Thus the heart tissues of the tadpoles resemble the heart tissues of the triton more closely, by their antigenic properties, than the heart tissues of the frog.

* The Table shows the mean values of the results of 79 tests.

TABLE 2

Results of the Complement Fixation Test Using Adsorbed Antisera to Frog's Heart with Antigens from the Heart of the Frog, Tadpole and Triton

Serum No.	Dilution	Antigens from the heart of the		
		frog	tadpole	triton
3939	1 : 20	+++	++++	++++
	1 : 40	++++	+++	++
	1 : 80	++++	+	±
	1 : 160	++	h	h
	1 : 320	±	h	h
	1 : 640	h	h	h
1917	1 : 20	++++	++	+
	1 : 40	++++	+	±
	1 : 80	+++	h	h
	1 : 160	+	h	h
	1 : 320	h	h	h
	1 : 640	h	h	h
2119	1 : 20	++++	+	±
	1 : 40	++++	h	h
	1 : 80	++	h	h
	1 : 160	+	h	h
	1 : 320	h	h	h
	1 : 640	h	h	h

The results shown in Table 1 were obtained with unadsorbed sera. In order to exclude any possible effect of antibodies to species-specific protein in the experiments described above, we adsorbed the sera with liver tissue, washed free from formalin, from animals of the same species as those to saline extracts of whose hearts the sera were obtained. The antisera to the heart tissues of the frog failed to react after adsorption, in all the dilutions used (1:20, 1:40, 1:80, 1:160, 1:320 and 1:640), with extracts of the skeletal muscle, liver and kidney of the frog. They reacted with homologous antigen in a dilution of 1:160. We were unable to completely adsorb antiserum to the heart tissues of the triton.

We carried out tests, the results of which are shown in Table 2, with adsorbed antisera to the heart tissues of the frog and extracts of the heart tissues of the frog, tadpole and triton.

As it may be seen from Table 2, adsorbed antisera to the frog's heart (Nos. 3939, 1917 and 2119) reacted with homologous antigen in a dilution of 1:160, and with antigen from the heart tissues of tadpoles in dilutions of 1:80, 1:40 and 1:20 respectively. The same sera (Nos. 3939 and 1917) reacted with extract of the heart tissues of the triton in dilutions of 1:40 and 1:20 respectively. There was no reaction between the serum (No. 2119) and the extract of the heart tissues of the triton.

The results of the experiments with adsorbed antisera to the heart tissues of the frog and extracts of the heart tissues of the tadpole, frog and triton thus corresponded to those obtained with the analogous unadsorbed sera (see Table 1). The degree of the reaction between the sera and the extracts of heart tissues of the tadpole, frog and triton was thus determined by antigens specific for these tissues.

The results of our experiments showed that the heart tissues of tadpoles differed in their antigenic properties from the heart tissues of the frog, and more closely resembled the heart tissues of the triton than the heart tissues of the frog. It was also shown that the heart tissues of tadpoles taken in stages 0, I-III and IV were indistinguishable in their antigenic properties in our experiments.

TABLE 3

The Anaphylaxis Test in Guinea Pigs Sensitized with a Suspension of Heart Tissues of Tadpoles in Response to the Injection of an Extract of the Heart Tissues of the Frog, Triton and Tadpole

Guinea pig No.	Sensitization (subcutaneously)			Desensitization (intravenously)			Test of complete desensitization (intravenously)			Assaulting injection I (intravenously)			Test of complete desensitization (intravenously)			Assaulting injection II (intravenously)		
	antigen	dose, mg	reaction	antigen	dose, mg	reaction	antigen	dose, mg	reaction	antigen	dose, mg	reaction	antigen	dose, mg	reaction	antigen	dose, mg	reaction
126	Suspension of heart tissue of tadpole	16	—	Extract of heart tissue of the frog	60	++	Extract of heart tissue of the frog	60	±	Extract of heart tissue of the triton	60	—	Extract of heart tissue of the triton	.	.	Extract of heart tissues of tadpoles	60	++++
437	The same	16	—	The same	60	+++	The same	60	—	The same	60	—	The same	.	.	The same	60	++
385	» »	16	—	» »	60	+	» »	60	—	» »	60	—	» »	.	.	» »	60	+++
104	» »	16	—	» »	60	—	» »	60	—	» »	60	—	» »	.	.	» »	60	±
978	» »	50	—	» »	60	+	» »	60	—	» »	60	+	» »	.	.	» »	.	.
312	» »	50	—	» »	60	+	» »	60	—	» »	60	—	» »	.	.	» »	.	.
238	» »	50	—	» »	60	++	» »	60	—	» »	60	++	» »	.	.	» »	.	.
143	» »	50	—	» »	60	++	» »	60	—	» »	60	++	» »	60	—	» »	40	+
398	» »	50	—	» »	60	+++	» »	60	—	» »	60	+	» »	60	—	» »	60	+++
418	» »	50	—	» »	60	+	» »	60	—	» »	60	++	» »	60	—	» »	60	++
502	» »	16	—		.	.		.	.		.	.		.	.	» »	60	++
511	» »	16	—		.	.		.	.		.	.		.	.	» »	60	+++
118	» »	16	—		.	.		.	.		.	.		.	.	» »	60	++++
101		.	.		.	.		.	.		.	.		.	.	» »	60	—
112		.	.		.	.		.	.		.	.		.	.	» »	60	—
114		.	.		.	.		.	.		.	.		.	.	» »	60	—

In connection with the experimental results shown in Tables 1 and 2, the question arose whether the differences in the antigenic properties of the heart tissues of the heart of the tadpoles and frog depended on the presence in the heart tissues of the tadpoles of antigens specific for these tissues alone. It was also of interest to find out whether it was not on account of these stage-specific antigens that the resemblance between the heart tissues of the tadpoles and triton was expressed to a greater degree than that between the heart tissues of the frog and triton. For this purpose we used the anaphylaxis with desensitization test in guinea pigs. One group of guinea pigs was sensitized subcutaneously with a suspension of the heart tissues of tadpoles in a dose of 16 mg per guinea pig, and another group in a dose of 50 mg per guinea pig. On the 21st day the guinea pigs were desensitized with extract from the heart tissues of the frog and, after testing for complete desensitization, an assaulting injection of extract of the heart tissues of the triton was given. This assaulting injection served at the same time a desensitizing purpose, since after it was injected an extract of the heart tissues of tadpoles as a second assaulting injection. The results of this experiment are shown in Table 3.

As may be seen from Table 3, guinea pigs sensitized with a suspension of the heart tissues of tadpoles in a dose of 16 mg did not react in response to an assaulting injection of extract of heart tissues of the triton. In guinea pigs sensitized with a suspension of the heart tissues of tadpoles in a dose of 50 mg, severe anaphylactic shock was observed in response to the assaulting injection of extract of the heart tissues of the triton. Finally, in response to the second assaulting injection of extract of the heart tissues of tadpoles, well-marked signs of anaphylactic shock were observed once again in all the animals.

Analysis of the results obtained showed that in the guinea pigs sensitized with a suspension of the heart tissues of tadpoles and desensitized to antigen of the heart tissues of the frog, anaphylactic shock took place in response to the injection of extract of the heart tissues of tadpoles. The heart tissues of tadpoles therefore contain antigens which are absent from the heart tissues of the adult frog (stage-specific antigens). It could also be concluded from the results of this experiment that in animals desensitized to antigen of the frog's heart, an anaphylactic reaction was also observed in response to the injection of extract of the heart tissues of the triton. The stage-specific antigens of the heart tissues of tadpoles thus possessed a resemblance to the antigens of the heart tissues of the triton. These antigens were presumably present in the heart tissues of tadpoles in small amounts, for we found them only in those cases in which a comparatively large dose of tissue (50 mg per guinea pig) was used for sensitization. Finally, in accordance with the results obtained, signs of anaphylactic shock were again observed in the guinea pigs after desensitization to antigen from the heart tissues of the frog and triton, in response to the injection of extract from the heart tissues of tadpoles. The heart tissues of tadpoles thus contained stage-specific antigens also, which did not resemble the antigens of the heart tissues of the triton. These antigens were present in the heart tissues of tadpoles evidently in larger amounts, for it was sufficient to sensitize guinea pigs with a dose of 16 mg in order to detect them.

It therefore followed, in the first place, from the results of our experiments that the heart tissues of tadpoles differed considerably in their antigenic properties from the heart tissues of the frog; in the second place that this difference depended on the presence in the heart tissues of tadpoles of stage-specific antigens, which were absent from the heart tissues of the frog; in the third place that the heart tissues of tadpoles resembled more closely in their antigenic properties the heart tissues of the triton than the heart tissues of the frog; in the fourth place that the closer resemblance between the antigenic properties of the heart tissues of the tadpole and the triton than that between the heart tissues of the frog and the triton was due to the presence of stage-specific antigens in the tadpoles. The fact of discovery in tadpoles of antigens absent from the adult frog and similar to the antigens of another species of animal, lower in its organization, may be explained, we are inclinced to believe, as a manifestation of antigenic recapitulation. Finally, it followed from our experiments that the heart tissues of tadpoles also contained stage-specific antigens which had no resemblance to the heart tissue antigens of the triton.

The experiments thus showed that the interspecies relationships of the antigenic properties of larval tissues are quite different from those of the analogous tissues of the adult animal of the same species.

SUMMARY

The complement fixation test and the anaphylaxis reaction with desensitization were employed in guinea pigs. It was shown that the cardiac tissues of tadpoles differ from those of frogs by their antigenic properties (particularly as a result of the presence of the antigens in tadpoles, which are absent in the adult frogs, i.e.

of the stage-specific antigens). Cardiac tissues of tadpoles are more similar by their antigenic properties to the cardiac tissues of Triturus taeniatus than the cardiac tissues of frogs at the expense of the stage-specific antigens, which are similar to the cardiac antigens of Triturus taeniatus. Stage-specific antigens with no similarity to the antigens of cardiac tissues of Triturus taeniatus were revealed in the cardiac tissues of tadpoles.

LITERATURE CITED

[1] R. F. Averkina, Byull. Éksptl. Biol. i Med. 41, 2, 70-73 (1956).*

[2] A. Boyden and G. K. Noble, Am. Museum Novitates. 606, 1 (1933).

[3] H. Braus, Arch. Entwickl. Mech. Org. 22, 564 (1906).

[4] R. M. Clayton, Nature, 168, 120 (1951).

[5] R. M. Clayton, Embryol. a. Exper. Morph. 1, 25 (1953).

[6] R. A. Flickenger and G. W. Nace, Exper. Cell Res. 3, 393 (1952).

[7] I. L. Krichevskii, Zbl. Bakt. 72, 81 (1914).

[8] G. H. Nuttall, Blood Immunity and Blood Relationship, New York Press, 1904.

[9] Cited by Philippson and G. H. Nuttall, 1904.

[10] J. L. Spar, J. Exper. Zool. 123, 467 (1953).

[11] P. Uhlenhuth, Haendel. Ergebn. wissenschaft. Med. 2, 1-4 (1910).

[12] P. Uhlenhuth and K. Wurm, Ztschr. Immunitätsforsch. 96, 183 (1939).

[13] K. Wilkoewitz and H. Ziegenspeck, Botanisch. Arch. 22, 227 (1928).

*Original Russian pagination. See C. B. Translation.

THE USE OF CORTISONE AND OF LONG-ACTING ACTH TO ASSESS THE BIOLOGICAL ACTIVITY OF THE SOMATOTROPIC HORMONE (GROWTH HORMONE)

I. A. Éskin and N. V. Mikhailova

From the Division of Experimental Biology (Head - Prof. I. A. Éskin)
of the All-Union Institute of Experimental Endocrinology
(Director - Prof. E. A. Vasyukova)

(Received April 25, 1958. Presented by Active Member AMN SSSR V. N. Chernigovskii)

The stimulating effect of the somatotropic hormone of the anterior lobe of the hypophysis in growth is based on its power to stimulate protein metabolism. As an anabolic hormone, somatotropin acts on every organ, which distinguishes it from the other tropic hormones of the hypophysis which have a selective action on a particular organ (a gland of internal secretion), but which makes its biological assessment difficult.

Many methods have been suggested for the biological assay of somatotropin. Some of these are based on the study of certain biochemical changes arising under the influence of the growth hormone (a fall in the non-protein nitrogen, urea, or amino acid content of the blood in dogs [13, 27, 28], a fall in the concentration of glutathione in the liver [16], an increase in the alkaline phosphatase or phosphorus content of the serum of hypophysectomized rats [23], retention of nitrogen [4, 14], an increase in the inclusion of sulfur from labled methionine in the skeletal muscles [11], etc).

Other methods are based on the stimulation of the body weight of normal rats in which the natural growth has been retarded and a so-called plateau established [10, 25], the stimulation of increase in weight of hypophysectomized rats [9, 24], the increase in the body weight of dwarf mice [12] and the increase in the length of the tail of hypophysectomized rats [8].

The most widely used method at the present time for the quantitative estimation of the biological activity of the somatotropic hormone is the "tibia test".

From examination of the development of the proximal epiphyseal cartilage of the tibia in normal and hypophysectomized rats it has been shown [21, 26] that after removal of the hypophysis the width of the cartilaginous epiphyseal plate is reduced and the equilibrium between the formation of cartilage and bone during endochondral ossification is disturbed. In the normal rat [5] the reduction in the thickness of the cartilaginous plate takes place mainly between the 40th and 65th day of life, and it subsequently proceeds at a slower rate. Removal of the hypophysis of 25-30 day-old rats leads to a rapid decrease in the width of the epiphyseal cartilage, and on the 14th day after operation this becomes constant. In response to injection of growth hormone an increase in the width of the cartilaginous epiphyseal plate of the tibia was observed in hypophysectomized rats [22]. On the basis of the data given above, a method of estimation of the biological activity of the growth hormone was developed, using 26-28 day-old female rats from which the hypophysis was removed [17]. From 12-14 days after hypophysectomy the rats were injected intraperitoneally for a period of 4 days with somatotropic hormone.

On the day after the last injection the rats were killed and, after suitable staining, the width of the epiphyseal cartilage of the tibia was measured under the microscope. The test was more sensitive than all

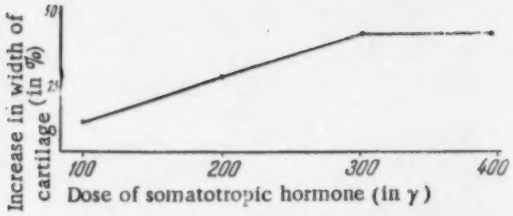


Fig. 1. Relationship between the increase in width of the epiphyseal cartilage of the tibia and the dose of somatotropic hormone.

other methods of determination of the biological activity of the growth hormone and did not take a long time (18 days).

It may, however, be carried out only on hypophysectomized animals, which introduces great difficulties in serial testing of the preparation, especially on a commercial scale. For this reason many workers have tried to find a method of determination of the biological activity of the somatotropic hormone in which hypophysectomy was unnecessary [1, 2, 7, 19].

The Effect of Methylthiouracil (30 mg per day) on the Body Weight and the Width of the Epiphyseal Cartilage

Duration of administration of MTU in days	Mean increase in body weight in g		Mean width of epiphyseal cartilage (in μ)	
	control	MTU	control	MTU
20	37	34	185	211
26	79	44	232	226
30	48	28	200	190
40	74	52	258	217
55	133	62	151	187
60	135	90	204	281
60	112	58	109	132
75	149	47	94	83

Our aim was to devise a method of testing the growth hormone in which the growth of the epiphyseal cartilage of the tibia could be inhibited without recourse to hypophysectomy. We took as our starting point reports in the literature and our own findings [3] that the action of ACTH and the corticosteroids was catabolic in character and that these preparations inhibited growth.

Becks, Simpson, Li and Evans [6] observed a reduction in the thickness of the epiphyseal cartilage after administration of ACTH. Huble [20] showed that cortisone markedly inhibits chondrogenesis in cockerels in the zones of proliferation (epiphyseal plate) of the proximal and distal ends of the femur, tibiotarsus and tarsometatarsus. Geschwind and Li [15] observed antagonism between hydrocortisone and the somatotropic hormone. After injecting 24 day-old female rats simultaneously with hydrocortisone and different doses of growth hormone, these authors observed marked inhibition of the increase in width of the epiphyseal cartilage of the tibia compared with that taking place in normal animals in response to somatotropin.

Our aim was to find out, in the first place, whether it was possible by the use of cortisone or ACTH to inhibit chondrogenesis in the epiphyseal cartilage of the tibia in female rats, and to obtain in a short period of time a reduction in the width of the epiphyseal cartilage of roughly the same degree as after hypophysectomy, and in the second place, whether in such rats stimulation of chrondrogenesis takes place in the epiphyseal cartilage of the tibia under the influence of growth hormone.

EXPERIMENTAL METHOD AND RESULTS

The effect of cartisone was investigated in 120 female rats weighing 30-35 g. On each of four successive days, 85 rats were injected with 1.5-2.0 mg of cortisone, and 35 rats received 0.05 mg daily for 14 days. The cortisone was injected once a day, subcutaneously. In all the experiments the cortisone inhibited the prolifera-

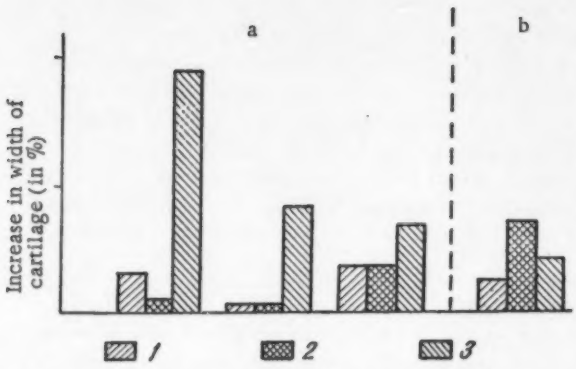


Fig 2. The effect of combined injection of somatotropic and thyrotropic hormones on the width of the epiphyseal cartilage of the tibia.
a) Administration of thyrotropic hormone in a dose of 400 γ; b) administration of throtropic hormone in a dose of 75 γ; 1) thyrotropic hormone; 2) somatotropic hormone; 3) somatotropic and thyrotropic hormones.

ation of the epiphyseal cartilage of the tibia and caused a reduction in its width. When 1.5 mg of cortisone* was injected daily for 4 days, the reduction in the width of the cartilaginous plate was 92-302 μ, or 23-63% of the control values, with an average of 41%. Injection of 0.5 mg of cortisone daily for 14 days caused a reduction in the width of the epiphyseal cartilage by 23-147 μ, or 8-39% of the control values, an average of 26%. The first method of injection of cortisone thus caused a more marked reduction in the width of the cartilaginous plate of the tibial epiphysis than the second. We compared the effect of injection of cortisone with that of removal of the hypophysis. The reduction in the width of the cartilaginous plate on the 14th day after hypophysectomy was on the average 49%, and after daily injection of 1.5 mg of cortisone for 4 days, it was 41%.

A similar although weaker effect was observed after the use of ACTH-zinc phosphate, i.e. a long-acting preparation (10-20 units daily for 4 days).

The width of the cartilaginous plate was reduced by 72-207 μ, i.e. by 21-44% of the control values, on the average by 32% (results for 45 rats). It must be emphasized that ACTH of the ordinary variety of action had no effect on the proliferation of the epiphyseal cartilage of the tibia, which was probably due to the fact that this preparation was rapidly excreted from the body.

We had at our disposal several samples of somatotropic hormone (STH), prepared in biochemical and experimental production laboratories of the All-Union Institute of Experimental Endocrinology, which contained insignificant traces of thyrotropin (less than 75 γ/mg STH). When injected intraperitoneally in a daily dose of 75 and 100 γ for 4 days to 100 intact rats, in which proliferation of the epiphyseal cartilage was inhibited by cortisone, STH stimulated growth of this cartilage and increased the width of the epiphyseal plate by 49-135 μ (400 γ STH) and by 57-187 μ (300 γ STH), i.e. on the average by 87 μ. The width of the epiphyseal cartilage was thus restored on the average by 50%. Similar results were obtained by the action of STH on 30 rats receiving ACTH-zinc phosphate. The results obtained were compared with the results following administration of a highly purified preparation of growth hormone, standardized in international units. 300-400 γ of such a highly purified STH also stimulated growth of the epiphyseal cartilage of the tibia on the average by 50% compared with control animals receiving cortisone.

We investigated the effect of various doses of STH from 100 to 800 γ (total dose) on the increase in width of the epiphyseal cartilage. With an increase in the dose of STH from 100 to 300 γ, a direct relationship was observed between the increase in width of the cartilaginous plate of the tibia in rats receiving cortisone and the dose of STH. Increasing the dose to 400γ and above was not accompanied by any further increase in the growth of the cartilage (Fig. 1).

On the basis of the results obtained we considered it possible to use intact female rats, weighing 30-35 g, for the biological assay of growth hormone, by replacing the complicated operation of hypophysectomy by injection of cortisone or ACTH-zinc phosphate according to the method described as follows.

Cortisone is injected subcutaneously in a single daily dose of 1.5-2.0 mg for 4 days. After the injections of cortisone have come to an end, STH is injected intraperitoneally in a daily dose of 75 or 100 γ for 4 days. This daily dose is given as three separate injections. The total dose of STH is 300-400 γ. 24 hours after the last injection of STH (the 9th day after the start of the test) the animals are killed and one or both tibias are removed, freed from soft tissues, split longitudinally and quickly stained or fixed in 10% formalin, the further treatment of the bone being according to the method described by Greenspan, Li, Simpson and Evans [17, 18].

* Cortisone from the firms of Merck, Roussel and Biddle Sawyer.

Before staining, the specimens are washed in water for half an hour, transferred to acetone for 1 hour and then again washed in water for half an hour. The bone is next placed in a freshly prepared 2% solution of silver nitrate for $1\frac{1}{2}$-2 minutes, rinsed in water, and exposed in water to the action of sunlight or illuminated with a clear electric lamp until the calcified areas stain a dark brown color. The stained halves of the bone are immersed for 25-30 seconds in 10% hyposulfite, rinsed in tap water for half an hour and allowed to stand in 80% ethyl alcohol in the dark. The width of the cartilaginous plate of the proximal epiphysis is measured by means of an ocular micrometer under low-power magnification of the microscope. Not less than 10 measurements are made of each half of the bone, and these values are converted into microns. Where cortisone is unobtainable it may be replaced by ACTH-zinc phosphate (10-20 units once a day, subcutaneously, for 4 days).*

We also investigated the possibility of using methylthiouracil (MTU) as an inhibitor of growth of the epiphyseal cartilage of the tibia [1]. In 8 series of experiments we gave 30 mg of MTU daily with the diet for 20-75 days. Besides complete failure of the animals to gain weight, or even loss of weight, in accordance with the reports in the literature [1, 2], we observed that the width of the epiphyseal cartilage was small. When we compared the width of the epiphyseal cartilage in 80 rats receiving MTU and 80 control animals of the same age, however, we found that the reduction in the width of the epiphyseal cartilage was not only the result of the action of MTU but also was in consequence of the retardation of growth due to age. In control animals not receiving MTU, the cartilaginous plate was roughly of the same width as in animals of the same age exposed to the action of MTU (see Table).

The failure of the animals receiving MTU to gain weight, associated with absence of changes in chondrogenesis in the tibial epiphysis, is probably evidence of the reduction in body weight of the rats in which the thyroid gland was blocked chemically being due not only to inhibition of true growth but also to disturbance of metabolism caused by exclusion of the function of the thyroid gland. Injection of STH in a total dose of 400 γ to rats both receiving and not receiving MTU increased the width of the epiphyseal cartilage on the average by 40 μ.

In conclusion we consider it necessary to draw attention to the importance of contamination of the STH preparation with thyrotropic hormone. From our observations, which are in agreement with findings in the literature, thyrotropic hormone in a dose of 400 γ has an insignificant effect on the proliferation of the epiphyseal cartilage (19 μ). However, after the simultaneous injection of 400 γ of thyrotropic hormone and 100 γ of STH (i.e. an ineffective dose), a significant increase in the width of the cartilaginous plate of the proximal tibial epiphysis was observed (Fig. 2). In other words, synergism exists between the thyrotropic and somatotropic hormones, which interferes with the objective assay of the growth hormone by means of the tibial test. After the simultaneous injection of small doses of thyrotropic hormone (75 γ) with STH, no synergism was observed in relation to the growth effect. For a correct estimation of the biological activity of STH, it is therefore essential to make allowance for the content of thyrotropic hormone therein. Only when the contamination by the latter is insignificant (75 γ/mg STH) can the action of the growth hormone, qualitative and quantitative, be objectively assessed.

SUMMARY

A method of evaluating the biological effect of the somatotropic hormone is given. It is based on the ability of the growth hormone to stimulate the proliferation of the epiphyseal cartilage of rat's tibia. The inhibition of the proliferation of the epiphyseal cartilage is reached by the subcutaneous administration of cortisone for 4 days (1.5 mg once a day). Subsequent injections of the somatotropic hormone (75-100γ daily for 4 days) reestablish the width of the epiphyseal cartilage to 50% on the average. In the absence of cortisone it may be replaced by ACTH-zinc-phosphate (10 units subcutaneously once a day for 4 days). The usual ACTH cannot be employed for this purpose.

* When our research had been completed, the abstracting journal "Biologiya" [No. 6, 1957] published an abstract No. 24314 of the paper by P. Sforzini, M. Negri and A. Mazzarella, from which it was gathered that these workers had successfully used large doses of ACTH to suppress growth of the epiphyseal cartilage of the tibia and had subsequently stimulated growth by means of growth hormone.

LITERATURE CITED

[1] N. K. Demokidova and M. Ya. Kabak, Problemy Éndrokrinol. i Gormonoterap., 3, No. 2, 111-113 (1957).

[2] M. Ya Kabak and E. B. Pavlova, Doklady Akad. Nauk SSSR, 67, No. 5, 945-948 (1949).

[3] I. A Éskin, Problemy Éndokrinol. i Gormonoterap. 1, 1, 52-59 (1955).

[4] P. D. Bartlett and O. H. Gaebler, Endocrinology 43, 329-335 (1948).

[5] H. Becks, M. E. Simpson and H. M. Evans, Anat. Rec. 92, 109-119 (1945).

[6] H. Becks, H. W. Simpson, C. H. Li et al., Endocrinology 34, 305-310 (1944).

[7] G. Dalfino, Folia endocrinol. 10, 317-326 (1957).

[8] E. Dingemanse, I. Freud and I. E. Uyldert, Acta Endocrinol. 1, 71-96 (1948).

[9] H. M. Evans, N. Uyei, Q. R. Bartz and M. E. Simpson, Endocrinology 22, 483-492 (1938).

[10] H. M. Evans, M. E. Simpson and C. H. Li, Growth 12, 15-32 (1948).

[11] F. Friedberg and D. M. Greenberg, Arch. Biochem. 17, 193-195 (1948).

[12] P. Fuss-Beck, Acta pharmacol. Toxicol. 3, 3 (1947).

[13] O. H. Gaebler, J. Exper. Med. 57, 349-363 (1933).

[14] O. H. Gaebler and W. H. Price, J. Biol. Chem. 121, 497-506 (1937).

[15] J. Geschwind and C. H. Li, International Symposium the Hypophyseal Growth Hormone, Nature and Actions, London 1955, 28-53.

[16] H. Goss and P. W. Gregory, Proc. Soc. Exper. Biol. Med. 32, 681-683 (1935).

[17] F. S. Greespan, C. H. Li, M. E. Simpson et al., Endocrinology 45, 455-463 (1949).

[18] F. S. Greenspan, et al., C. W. Emmens', Hormone Assay, New York 1950, 73.

[19] M. Griffiths and F. G. Joung, J. Endocrinol. 3, 96-106 (1942).

[20] J. Huble, Acta Endocrinol. 25, 59-63 (1957).

[21] T. H. Ingalls, Endocrinology, 29, 710-724 (1941).

[22] E. A. Kibrick, H. Becks, W. Marx et al., Growth, 5, 437-447 (1941).

[23] C. H. Li, I. Geschwind and H. M Evans, Endocrinology 44, 67-70 (1949).

[24] C. H. Li, H. M Evans and M. E. Simpson, J. Biol. Chem. 159, 353 (1945).

[25] W. Marx, M. E. Simpson and H. M Evans, Endocrinology 30, 1-10 (1942).

[26] R. D. Ray, H M. Evans and H. Becks, Am. J. Path. 17, 509-528 (1941).

[27] H. M. Teel and H. Cushing, Endocrinology 14, 157-163 (1930).

[28] H. M. Teel and O. Watkins, Am. J. Physiol. 89, 662-685 (1929).

MORPHOLOGY AND PATHOMORPHOLOGY

DATA ON THE INNERVATION OF THE MUCOUS MEMBRANE OF THE STOMACH IN MAMMALS AND MAN

F. D. Gilev

From the Department of Histology (Head - Prof. G. A. Nevmyvaka)
of the Perm Medical Institute

(Received March 30, 1957. Presented by Academician L. A. Orbeli)

Despite the large volume of research devoted to the study of the innervation of the alimentary tract, we still do not have sufficiently complete morphological data on this subject.

In relation to the innervation of the stomach in mammals, the descriptions in the literature deal with mainly the subserous, intermuscular and submucous plexuses and the ganglia contained within them. Contemporary workers have shown that the cells of the first type, discovered by A. S. Dogel', predominate in the ganglia of the stomach, and the cells of the second type are present in that organ in relatively small numbers [2, 4 and others].

There are very few references in the literature to the innervation of the mucous membrane itself in the stomach. In a paper by K. A. Kytmanov [3] there is mention of a plexus in the mucous membrane, and drawings are given of nerve endings on the chief and parietal cells. K. A. Lavrov [5] observed small nerve ramifications among the cells of the gastric glands. A paper by Greving and Berg [6] has appeared in recent years, in which the presence of a nerve plexus containing solitary nerve cells and ganglia is indicated in the muscular coat of the mucous membrane.

EXPERIMENTAL METHOD

Our experimental material consisted mainly of young dogs, and partly of cats. As our principal method we used staining with methylene blue, by means of injection of the dye into the vascular system after preliminary flushing of the vessels with warm physiological saline. Fixation was with ammonium molybdate solution. The nerves were studied mainly in total preparations, but thick sections were cut from part of the material with a freezing microtome.

EXPERIMENTAL RESULTS

In total preparations and also in thick sections it could be seen that the mucous membrane of the stomach of dogs and cats was supplied with nerves from the submucosa. Thick bundles of nerve fibers, emerging from the submucous plexus, entered the muscular coat of the mucous membrane, and there ramified. The diverging branches extended along the outer surface of the muscular layer of the mucous membrane, sometimes for quite a long distance, and then turned inwards, perforating the muscular layer and entering the mucous membrane proper (t. propria). Here, these quite thick bundles of nerve fibers ramified and formed a nerve plexus, composed of loops of different sizes, situated immediately beneath the peptic glands. Some idea of the structure of this plexus may be obtained from an examination of Fig. 1.

Along the course of the bundles in the plexus of nerve fibers were seen nerve cells disposed singly. These comparatively large, multipolar cells usually were poorly stained and hence were found with great difficulty. From the body of these cells emerged several processes, which sometimes could be traced for a considerable

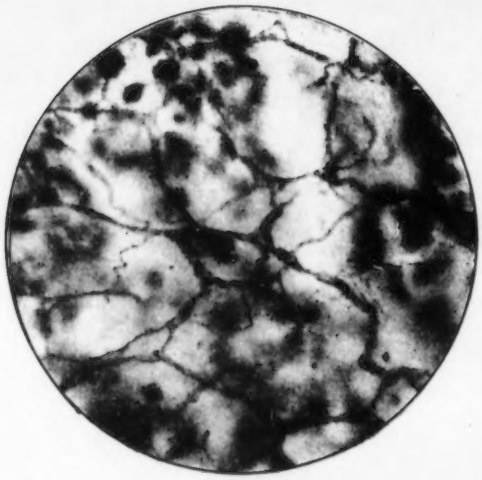


Fig. 1. Portion of a nerve plexus from the mucous membrane of the stomach of a puppy. Total preparation. In preparing this specimen the mucosa was separated from the remaining parts of the stomach wall. Appearance from the outer surface (t. m. m. removed). Microphotograph.

Fig. 2. Ganglia from a nerve plexus in the t. m. m. of the mucous membrane of the human stomach. Total preparation. In preparing this specimen the outer parts of the stomach wall were removed. Microphotograph.

distance. By their morphological character, these cells were most likely to be type two Dogel' cells.

Bundles of nerve fibers, forming yet another plexus in the mucous membrane, left this plexus in the direction of the inner surface; they were situated directly between the gastric pits and consist of bundles of slender fibers. From the bundles of this plexus branched out slender fibers in the direction of the epithelium lining the surface of the stomach and the gastric pits. At the bases of the epithelial cells these fibers divided

Fig. 3. Portion of a nerve plexus from the mucous membrane of the human stomach. Total preparation. Stained with methylene blue. In preparing this specimen the outer layers of the stomach wall were removed. Seen from the outer surface (t. m. m. removed). Microphotograph.

and terminated either freely or in pin-head thickenings. These endings were mainly situated at the junction of the epithelium with the connective tissue. Some of these fibrils penetrated the epithelium and terminated as expansions on the surface of the epithelial cells.

We thus observed two plexuses in the mucous membrane of the stomach in dogs and cats: one – on the surface of the mucous membrane, at the level of transition of gastric pits into glands, and a second, deeper one – between the base of the gastric glands and the muscular layer of the mucous membrane. From these plexuses and from the bundles of nerve fibers connecting them, were given off slender lateral fibrils, supplied with varicose thickenings. These fibrils formed numerous nerve endings both in the connective tissue and in the epithelium itself. By their situation and morphological structure, the nerve endings could be classified as receptor, responsible for the production of afferent impulses from the mucous membrane of the stomach, although some of them, situated in the immediate vicinity of the glands, were possibly effector in nature, and concerned with the regulation of the secretory function of the gland cells. However we were unable to find any clearly marked morphological differences between them.

We also obtained some information on the innervation of the mucous membrane of the human stomach. The material used for this was areas of human stomach removed at operation. This material was made available to us by Prof. N. M. Stepanov, Head of the Surgical Clinic of the Perm Medical Institute. Specimens were prepared from the peripheral areas of the excised material, i.e. from places where the wall of the organ had preserved a more or less normal structure. We used methylene blue for staining here also. As a preliminary step the mucous membrane was mechanically separated from the remaining portions of the stomach wall. The specimens were stained in the incubator and periodically moistened with a weak solution of methylene blue. After fixing in the ammonium molybdate solution and rinsing, the mucosal layer was stripped from the specimens, after which total surface preparations were obtained from both separated layers, and also preparations in which the mucous membrane remained attached.

In the preparations thus obtained, thick bundles of fibers could be seen penetrating into the mucous membrane from the submucosa. Along their path in the muscular layer of the mucosa these bundles ramified and formed a nerve plexus, situated between the layers of muscles. The loops of the plexus were formed of comparatively thick bundles of nerve fibers and were of different shapes and sizes. Along the course of the bundles and in the ganglionic areas, especially between the internal and middle layers of muscles, nerve cells

were found in the plexus, both solitary and in whole groups; in the latter case they formed true ganglia. One such ganglion is illustrated in Fig. 2. The nerve cells here were of different sizes, and all were multipolar. Their processes could sometimes be traced for a considerable distance.

We saw a similar plexus in the muscular coat of the mucous membrane also in preparations from the stomach of dogs, but there we were unable to detect any ganglia, finding only solitary nerve cells along the course of the bundles of nerve fibers.

Nerve endings were also found in the muscular coat of the mucous membrane of the human stomach. The nerve fibers forming these endings were supplied with numerous varicose thickenings of various shapes. The endings themselves consisted of interlacing, thin nerve branches, somewhat reminiscent of glomeruli in their form. We found no capsule around these glomeruli.

Bundles of nerve fibers penetrating from the submucosa into the mucosa proper (t. propria) formed here a plexus which was situated at the level of the base of the gastric glands; the thicker bundles here were distributed mainly in the intervals between the glands and formed here wide loops inside which a network of thinner fibers could be seen. Thanks to this, the base of each gastric gland (sometimes two together) was lightly enveloped in a peculiar type of alveolus formed by the nerve plexus. The edge of this alveolus was formed from loops of thick bundles of the plexus, and its middle part – of a network of finer fibers. One portion of such a plexus is shown in Fig. 3. In this plexus too, along the course of the nerve bundles, nerve cells were seen – singly or in groups of two or three.

Nerve endings were observed here also. They were formed from slender nerve fibers branching from the bundles of the plexus and situated in the connective tissue between the glands. The usual form of the endings was that of a glomerulus, formed by interlacing of a profusely coiled thin nerve fibril. Along the course of the fibril were seen numerous varicose thickenings of various shapes. Some of these glomeruli were greatly elongated, while others were more round in form.

We pointed out above that two nerve plexuses were noted in the t. propria of the mucous membrane of the stomach of dogs: one – at the base of the gastric glands, the other – at the level of transition from gastric pits into glands. In the t. propria of the human stomach we have so far been able to find only one plexus – at the base of the gastric glands. In the cases which we have examined, the bundles of this plexus in the human stomach were wider and more friable, thanks to which the whole plexus had the character of a comparatively dense network; in dogs the bundles were distributed more sparsely and they were thinner. These differences were sufficiently prominent from a comparison of Fig. 1 and 3 (microphotographs taken with the same magnification).

We have so far been able to examine only a small number of human stomachs (seven cases altogether), whereas in studying the innervation of the stomach in dogs we have dealt with over 300 animals. The fact that the nerve plexus in the mucous membrane of the human stomach at the transition point between gastric pits into glands has not been detected may possibly be explained by the small number of cases studied.

SUMMARY

The author stained the nerves with methylene blue and revealed nerve plexuses both in its mucous membrane (where ganglia were also found, especially in man), and in the t. propria. In man such plexuses are located at the floor of the glands. There are additional plexuses in cats and dogs – on the level of transition of the gastric fossae into glands. These plexuses have fibers with ramifications which form numerous nerve endings in the connective tissue and at the bases of the epithelial cells.

LITERATURE CITED

[1] A. S. Dogel', Anat. Anz. 11, (1896).

[2] N G. Kolosov, Trudy Tatarsk. Nauchno-Issled. Inst. Teor. i Klin. Med. 2, (1935).

[3] K. A Kytmanov, Nevrol. Vestnik, 5, 2 (1897).

[4] B. I. Lavrent'ev, Morphology of the Autonomic Nervous System. Collected Works, 1939. [in Russian].

[5] K. A. Lavrov, The Terminal Divisions of the Peripheral Nervous System, 1941. [in Russian].

[6] R. Greving and G. Berg, Acta Neuroveget. 8, 3 (1954).

CHANGES IN THE LUNG AFTER LIGATION OF THE PULMONARY ARTERY AND INTRODUCTION OF A FOREIGN BODY INTO THE TRACHEA

N. V. Voikova

From the Department of Pathological Anatomy (Head – Prof. M. A. Zakhar'evskaya) of the I. P. Pavlov First Leningrad Medical Institute

(Received December 29, 1957. Presented by Active Member AMN SSSR N. N. Anichkov)

The changes in the lungs after ligation of the pulmonary artery began to be studied at the beginning of the twentieth century. Experimental investigations showed [21, 22, 11, 12, 19, 4, 13] the development of pneumosclerosis, the genesis of which remained uncertain. In addition the formation of bronchiectasis was observed, and in individual cases authors spoke out against the use of this operation in bronchiectasis [12, 13]. Some surgeons recognize the practical importance of the operation as an independent event, improving the general condition of the patient and leading to a reduction in the inflammatory manifestations in the lung [9, 1, 3, 14, 15, 16, 17, 18].

In view of the contradictory experimental and clinical findings and also of the inadequate number of systematic morphological investigations, we considered it timely to carry out an experimental investigation to study the changes in the lung tissue after ligation of the pulmonary artery; under these circumstances we directed our attention not only to the results of ligation in the course of their development but also to the special features of the course of the nonspecific inflammation arising in such cases.

EXPERIMENTAL METHOD

We carried out 2 series of experiments on rabbits weighing from 1.5 to 2.5 kg. In the first series of 26 rabbits the pulmonary artery was ligated, and in the second series of 16 rabbits, after ligation of the pulmonary artery a foreign body was introduced into the trachea. The pulmonary artery was ligated transpleurally; in conformity with existing indications [18] the artery was not divided. The rabbits were killed by air embolism at periods from 12 hours to 12 months. After the chest was opened, the trachea was ligated. The lungs were fixed in the opened pleural cavity in 10% formalin solution; after fixation, total preparations were made from each lobe of both lungs. The material was embedded in paraffin wax, and short series of sections (8-10) prepared from the lungs; from 15 to 200 sections were made from the trachea in the second series of experiments.

The following stains were used: hematoxylin-eosin, picrofuchsin by Van Gieson's method, the same method in conjunction with fuchsilin (for elastic tissue), mucicarmine, sometimes by Heidenhain's iron-hematoxylin, by the Gram-Weigert method, and in some cases by Foot's silver impregnation method.

EXPERIMENTAL RESULTS

In the first series of experiments no morphological changes were found in the right lung on macroscopic and microscopic examination. Both lobes of the left lung in the early stages were dark red in color, and later friable adhesions were present between them and the parietal pleura. Starting on the 10th day, the lung was reduced in size; its tissue was airless or rose-colored, and flabby. In the experiment, which lasted 12 months, the formation of bronchiectases was observed.

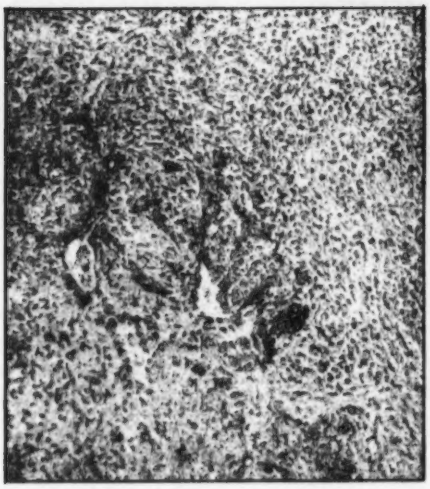


Fig. 1. Bands of indifferent epithelium from small caliber bronchi, in the form "whiskers." Stained with hematoxylin-eosin. First series of experiments. Time of experiment 7 days. Magnification: objective 16 ×, ocular 9 ×. (Zeiss).

Fig. 2. Proliferation of connective tissue in the walls of alveoli. The alveolar epithelium is in the form of layers of flattened and cubical cells. Van Gieson's stain. First series of experiments. Time of experiment 8 days. Magnification: ocular 6 ×, objective 16 × (Zeiss).

On microscopic examination it was found that after 12-24 hours the tissue of the left lung was aerated and in a condition of evenly distributed hyperemia; in places small hemorrhages were observed. On the 2nd and 3rd day, in addition, some pallor in the staining of the alveolar tissue could be noted. Later, on the 6th, 7th, 8th, 13th, 14th, 35th, 46th, 75th and 103rd days, an anemic infarct was found and this subsequently underwent organization.

The necrotic foci differed in size. Usually the center of the lobe became necrotic, occasionally the whole lobe; under these circumstances the wall of the main bronchus was often included in the necrosis. In the region of the focus of necrosis the cell muclei did not stain. The collagen fibers stained a pale color and their structure was indistict. The muscular coat of the vessels and bronchi appeared as a homogeneous mass, devoid of nuclei; the elastic and argyrophilic framework of the lung was preserved. Beneath the pleura a variable amount of lung tissue was preserved. The vessels in this zone had walls of the usual structure, and were filled with blood. Bands of connective tissue with large numbers of stretched-out blood vessels grew out toward the outer surface of the pleura. Around the foxus of necrosis, leucocytes formed a powerful barrier. Starting on the 8th day, outside the leucocyte barrier proliferation of connective tissue was observed, especially pronounced around the main bronchus. In and among the connective

Fig. 3. Organization of the lung parenchyma in association with diffuse proliferation of connective tissue. Tubes of stratified squamous and high cylindrical epithelium with mucous and a few leucocytes in the lumen. Infiltration of the connective tissue between the tubes with cells. Stained with hematoxylin and mucicarmine. First series of experiments. Time of experiment 131 days. Magnification: ocular 4 ×, objective 8 ×. (Zeiss).

tissue were seen remnants of the elastic framework of the lung, and also complex formations and bands of indifferent epithelium, round or elongated in shape (Fig. 1). In some of the complex formations a lumen could be distinguished; their epithelium became cylindrical or prismatic. Side by side with these complex formations were usually seen tubes of a regular, round or oval shape, lined with the same epithelium. If the wall of the lobar bronchus was included in the necrosis, its epithelium spread under the necrotic tissue, separating it from the tissue which was preserved, and at the same time formed processes which infiltrated the surrounding young connective tissue in the form of branching tubules. Around the periphery of the necrotic focus could often be seen thickened areas of connective tissue in the walls of the alveoli, with a well-marked epithelial lining at early stages this lining consisted of flattened epithelium, often even in the form of separate cells, and later of cubical epithelium.

In 8 cases no necrotic changes could be found in the lung tissue, and only diffuse and focal proliferation of connective tissue could be distinguished. On the 69th, 118th and 131st days, diffuse proliferation of connective tissue was found, so that the lobes of the lung were converted into adenoma-like tissue. The tubes were lined with cylindrical and prismatic epithelium, producing mucus; the lumen of the tubes contained a small number of leucocytes (Fig. 2). Between the tubes was a small amount of connective tissue, infiltrated by lymphocytes and histiocytes. Among the proliferating connective tissue were found vessels, filled with blood, with walls consisting of an elastic membrane and connective tissue. No arteriovenous anastomoses nor occluded arteries could be observed.

Foci of proliferation of connective tissue were seen on the 21st, 91st, 174th, 183rd, and 211th days, in the form of wedge-shaped or narrow scars containing adenoma-like formations and vessels similar to those described above in the case of the diffuse proliferation of connective tissue. The lung tissue surrounding the cicatricial foci was unchanged and in places emphysematous and filled with air. Arteriovenous anastomoses and occluded arteries were often found. With both focal and diffuse proliferation of connective tissue, numerous vessels could be seen in the commissures.

In the experiment, which was continued for 12 months, the tissue infiltrating the lobes was converted into large cavities containing leucocytes and lined with stratified squamous epithelium.

Histological examination of the pulmonary artery in the region of the ligature (12 cases) showed obliteration of its lumen by connective tissue and elastic fibers. From this description it was thus clear that after ligation of the pulmonary artery an anemic infarct was produced, with subsequent organization of the necrotic mass. The size of the scar evidently depended on the area of the preceding necrosis. After extensive areas of necrosis, the proliferation of connective tissue was diffuse and led to complete organization of the lung; small areas of proliferation terminated in focal pneumosclerosis.

If we accept the well-known scheme of the circulation in the lung [20], with slight modifications [10], then some idea may be gained of the circulation in the lung during the development of focal pneumosclerosis after ligation of the pulmonary artery. Blood from the bronchial artery passed through a system of arterial shunts into branches of the pulmonary artery, and partly through arteriovenous anastomoses into the venous system.

In addition blood reached the lungs through the commissures and pleural vessels. The arterial shunts presumably fulfil the role of arterio-arterial anastomoses. In diffuse pneumosclerosis the system of arterial shunts and arteriovenous anastomoses evidently did not come into action; the surviving lung tissue obtained its blood supply through its own particular bronchial artery and through vessels in the commissures and the pleura.

The second series of experiments consisted of a combination of ligation of the pulmonary artery and the introduction of a foreign body into the trachea. The foreign body, in the form of a sterile silk thread, was introduced into the trachea on the 14th, 92nd and 212th day after the operation of ligation of the pulmonary artery. As controls we used our experiments in which a foreign body was introduced into the trachea, described in a separate communication. At the level of the foreign body inflammation developed in the wall of the trachea; leucocytes and Gram-positive cocci accumulated in the lumen around the thread. In three cases, focal pneumonia was found in the right lung, and in the remainder bronchitis and hyperplasia of the lymphoid structures of the bronchi.

In the left lung, with the ligated pulmonary artery, inflammation developed against the background of the changes described above. In those cases when the anemic infarct was not yet successfully organized (introduction of the foreign body on the 14th and 92nd day after ligation of the pulmonary artery), bronchiectasis developed in the lung tissue (3 cases, Fig. 3), abscesses (1 case) and sequestration of the necrotic mass with a wide leucocyte barrier at the margin of the necrotic area (4 cases). In the experiments in which the necrosis had undergone organization (3 cases in which the foreign body was introduced on the 92nd day and 5 cases on the 212th day after ligation of the pulmonary artery), the changes were either indistinguishable from those described in the first series, or they consisted of some increase in the number of leucocytes in the lumen of the adenoma-like formations.

The results of both series of our experiments thus showed the possibility of infection via the bronchus of lung tissue, changed after ligation of the pulmonary artery. Infection was possible after ligation of the pulmonary artery alone, and even more so when this was combined with a foreign body in the trachea. In the latter case the conditions were more favorable for infection and the process of organization was complicated by suppuration, and sequestration and bronchiectasis usually arose.

The entire reorganization of the lung parenchyma which we observed in both series of experiments was of considerable interest. In the literature are descriptions of proliferation of tubes of cubical epithelium, referred to as pseudobronchi [13]. We have not found any more detailed description of these changes in the epithelium. In our experiments we also found proliferation of bands of indifferent epithelium as well as tubes of cylindrical, prismatic and stratified squamous epithelium. Evidently this was primarily proliferation of the epithelium of the bronchi, but there were grounds for the view that the epithelium of the alveoli also became modified. Nevertheless proliferation of the bronchial epithelium, if an inflammatory and regenerative character, was predominant. In the region of the necrosis in the wall of the main bronchus, the epithelium grew in a layer which crept over the living tissue and separated it from the necrosis. This type of growth was regenerative, but at the same time it could also be regarded as inflammatory growth, thanks to which delineation of the necrotic mass from the living tissue took place [6, 7, 8].

We were able to observe complete overgrowth of an extensive focus of necrosis connected to the wall of the main bronchus or a lobar bronchus. It is possible that bronchiectasis also developed as a result of this overgrowth. Around the intrapulmonary bronchi of varying calibar were found areas of epithelial proliferation, in the form of layers of indifferent epithelium and tubes of cylindrical and prismatic bronchial epithelium. Complete regeneration of the bronchi did not, therefore, take place; only a system of tubes was formed, connected to the residual bronchi.

We observed a peculiar reaction of the epithelium of the alveoli. At early stages, during proliferation of loose connective tissue in the wall of the alveolus, the alveolar epithelium could be seen in the form of a layer of flattened epithelial cells; at later stages, during conversion of the connective tissue into scar tissue, the alveolar epithelium became cubical. This accomodation of the alveolar epithelium was associated with the interference with its function and also with the change in the state of the underlying tissue, i.e. it was the result of disturbance of the unity of the physiological system "epithelium - connective tissue" [7, 8, 5].

SUMMARY

Experiments with the ligature of the pulmonary artery were conducted in 26 rabbits. An anemic infarction with subsequent organization and development of focal and diffuse pneumosclerosis was obtained. Inflamatory and regenerative proliferation of the bronchial epithelium in the form of indifferent bands and the layer demarcating the necrosed tissue was observed together with the proliferation of the connective tissue.

Besides there is seen an appearance of the alveolar epithelium with its transformation into cubic epithelium. Combination of the pulmonary artery ligature with introduction of a foreign body into the trachea (16 rabbits) resulted in more intense inflammation. The development of bronchiectasis was noted in 2 cases and sequestration of necrosed masses – in 3.

LITERATURE CITED

[1] A. N. Bakulev and A. V. Gerasimova, Khirurgiya, 8, 50-61 (1952).

[2] N. V. Boikova, Problems of Morphology and Physiology 3, 221-227, Riga, 1958.*

[3] A. V. Gerasimove, Khirurgiya 7, 18-24 (1949).

[4] M. B. Dolgopol, Vestnik Khir. im. Grekova 17, 50 86-93 (1929).

[5] A. A. Zavarzin, Collected Works, 4, 348-389 Moscow - Leningrad, 1953.*

[6] V. G. Garshin and L. M. Shabad, M. Ztschr. f. Krebsforsch. 43, 137-145 (1935).

[7] V. G. Garshin, Inflammatory Proliferation of Epithelium, Its Biological Importance and Reaction to the Problem of Cancer, pp. 62-79, Moscow-Leningrad, 1939.*

[8] V. G. Garshin, Transactions of the Histological Conference, 150-155, Moscow, 1949.*

[9] I. I. Grekov, Transactions of the Sixteenth Congress of Russian Surgeons, 396-397, Leningrad, 1925.*

[10] A. V. Ryvkind, Arkh. Patol. 10, 3, 24-35 (1948).

[11] S. A. Smirnov, Vestnik Khir. im. Grekova 4, 12, 101-105 (1924).

[11b] S. A. Smirnov, Transactions of the Sixteenth Congress of Russian Surgeons, 382-383, Leningrad, 1925.*

[12] S. A. Smirnov, Arch. klin. Chir. 141, 512-518, 1926.

[13] I. G. Turovets and V. A. Grimailovskaya, Vrachebnoe Delo 8, 745-750 (1955).

[14] F. G. Uglov, Khirurgiya 6, 50-54 (1951).

[15] F. G. Uglov, Vestnik Khir. im. Grekova 68, 4, 22-28 (1948).

[16] F. G. Uglov, Vestnik Khir. im. Grekova 3, 57 (1948).

[17] F. G. Uglov, Sovet. Med. 6, 15-16 (1948).

[18] F. G. Uglov, Resection of the Lungs, 223-235, Leningrad, 1954.*

[19] K. N. Cherepnin, Vestnik Khir. im. Grekova 7, 19, 105-113 (1926).

[20] H. V. von Hayek, Ztschr. Anat. 110, 412-422 (1940).

[21] O. Bruns and F. Sauerbruch, Mitt. Grenzgeb. Med. Chir. 23, 343-350 (1911).

[22] K. Kawamura, Dtsch. Ztschr. Chir. 125, 373-384 (1913).

* In Russian.

METHODS

A METHOD OF DETERMINATION OF THE OXYGEN DEMAND OF CARDIAC MUSCLE BY MEANS OF THE KREPS OXIMETER

I. E. Kisin

From the Laboratory of Special Pharmacology (Head - Active Member AMN SSSR V. V. Zakusov) of the Institute of Pharmacology and Chemotherapy AMN SSSR, Moscow

(Received June 28, 1958. Presented by Active Member AMN SSSR S. E. Severin)

It can now be accepted as proved that the rate of the coronary blood flow is determined by the oxygen demand of the heart [3]. The relationship between the rate of the coronary blood flow and the oxygen demand of the heart has even been expressed mathematically [5]. Accordingly, a reasonably complete picture of the coronary circulation may be obtained if simultaneously with the study of the rate of the coronary blood flow, the oxygen demand of the heart is determined.

Usually in the study of the coronary circulation, in order to measure the oxygen demand of the cardiac muscle the oxygen content of samples of arterial and venous blood is measured, the blood being drawn simultaneously from the artery and the coronary sinus. The oxygen content of the blood samples is estimated by Van Slyke's method. This method is sufficiently accurate but it is very laborious. Neither does it allow continuous measurement of the oxygen content of the blood in the coronary sinus.

In the present paper is described a photoelectric method of determination of the oxygen demand of the heart muscle of the cat, based on the measurement of the content of oxyhemoglobin in the venous blood flowing from the coronary sinus.

The photoelectric method of continuous estimation of the oxygen content in unopened vessels was first described in 1935 by Kramer [4]. Later Drabkin and Schmidt [2] suggested determining the content of oxyhemoglobin in the arterial blood flowing through a receiver by means of a spectrophotometer. Several other papers [6, 7, 8] were devoted to the development of spectrophotometric methods of rapid determination of the oxygen content in blood samples obtained by catheterization of the various chambers of the heart.

For the continuous measurement of the oxygen content of the blood flowing from the coronary sinus we used the oximeter devised by E. M. Kreps. The Kreps apparatus was designed for determining the oxyhemoglobin content of the arterial blood. The detector of the apparatus is applied to the pinna of the ear, in which, after warming, the blood is almost completely arterial. So that the oxyhemometer could be used to determine the oxyhemoglobin in the blood flowing from the coronary sinus, a receiver made of organic glass and having flat sides was inserted into the detector of the apparatus. The lumen of the receiver was 1 mm deep. The receiver was joined on one side to a polyethelene catheter along which blood was brought from the coronary sinus, and on the other side, by means of a rubber tube, to the jugular vein (Fig. 1).

The Kreps oxyhemometer was designed to determine oxyhemoglobin between limits of 100 and 60%, but the oxyhemoglobin content of the blood flowing from the coronary sinus of the heart may reach 20%. In view of this, the scale of the apparatus was extended to the required limits, and recalibrated. For this purpose, by means of parallel determinations of the oxyhemoglobin content using the Kreps oximeter and the OKO-01 reflecting oximeter (the readings of the reflecting oximeter were first checked against those obtained by Van Slyke's method), a calibration curve was drawn up for the new scale of the Kreps oximeter.

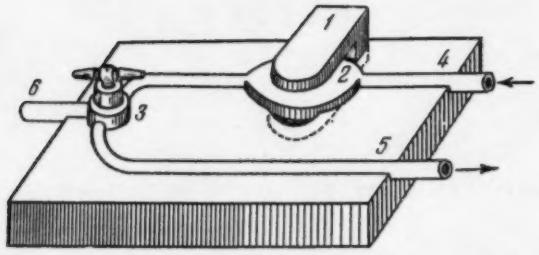


Fig. 1. Diagram of the apparatus for measuring the oxyhemoglobin content of the blood flowing from the coronary sinus.
1) Oximeter detector; 2) receiver; 3) cock; 4) tube bringing blood from the coronary sinus; 5) tube returning blood to the jugular vein; 6) tube leading to the apparatus for measuring the rate of the coronary blood flow.

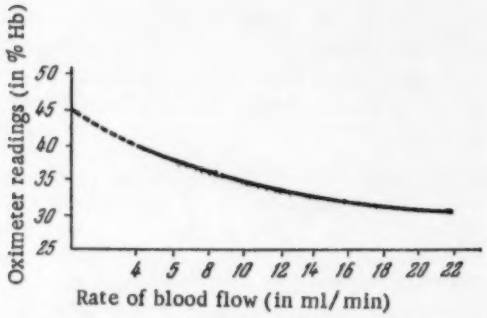


Fig. 2. The effect of the rate of blood flow on the readings of the oximeter.

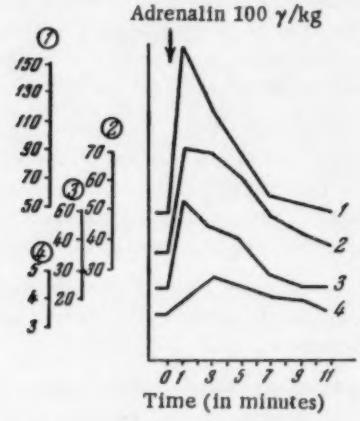


Fig. 3. The effect of adrenalin on the coronary circulation.
1) Arterial pressure in mm of mercury; 2) rate of blood flow from the coronary sinus in ml / min/ 100 g weight of the heart; 3) oxyhemoglobin content in the blood of the coronary sinus in %; 4) oxygen demand of the heart from the blood drained by the coronary sinus, in ml / min/ 100 g weight of the heart.

In view of the fact that during the determination of the degree of saturation of the hemoglobin with oxygen in the blood flowing through the receiver the rate of flow may alter, we investigated the relationship between the rate of flow of blood and the readings of the oximeter.

By means of a perfusion pump, which allowed the rate of the blood flow to be varied, the results shown in Fig. 2 were obtained. As may be seen from the Figure, with an increase in the rate of blood flow, the readings of the apparatus tended to show a lower percentage content of oxyhemoglobin. However, in view of the fact that the rate of blood flow from the coronary sinus of the heart of the cat is 4-8 ml/min, and the maximum increase in the rate of the blood flow does not usually exceed 50-100%, the error due to changes in the rate of blood flow is insignificant: an increase of up to 50% in the rate of blood flow will give an error not exceeding 2% of oxyhemoglobin, and an increase of up to 100% – not more than 3-4%. In order to obtain more accurate values of the oxygen saturation of the hemoglobin, the blood flow can be arrested for each determination of the oxyhemoglobin.

It has to be borne in mind that by means of the Kreps apparatus only relative values of the oxyhemoglobin can be obtained, i.e. the scale of the oximeter must be calibrated by testing blood with a known percentage content of oxyhemoglobin, and only then can readings be taken under the desired conditions. For this reason, at the beginning of each experiment the oxyhemoglobin content of the blood entering the receiver inserted into the detector of the Kreps oximeter was determined by means of a reflecting oximeter, after which the pointer of the apparatus was moved to the figure obtained. If no reflecting oximeter is available, a sample of arterial blood is oxygenated in the air for 15-20 minutes (in an open vessel, with agitation), which usually ensures complete saturation of the blood with oxygen. The sample of blood is then introduced into the receiver and the pointer of the oximeter is moved to 100% of oxyhemoglobin.

By the use of this method the percentage content of oxyhemoglobin in the outflowing venous blood was determined. In order to calculate the oxygen demand of the heart muscle, the following formula was applied:

$$A = \frac{1{,}34\ (C_1 - C_2)\ VH}{100},$$

where A is the quantity of oxygen in ml/min; C_1 is the oxyhemoglobin content in the arterial blood in per cent (determined by the reflecting oximeter; under the conditions of our experiments, with artificial respiration, it was constant within the limits of possible error of the apparatus of ±5%); C_2 is the oxyhemoglobin content in per cent in the blood flowing from the coronary sinus; V is the rate of flow of blood from the coronary sinus in min/min (this value was determined in parallel with the measurement of the oxyhemoglobin content by the method described in the paper by N. V. Kaverina [1]); H is the quantity of hemoglobin in 1 ml of blood, in g. The hemoglobin content was determined by means of a photoelectric erythrohemometer.

Figure 3 shows the results of an experiment on a cat, obtained by means of the method just described. In view of the fact that the coronary sinus drains mainly the blood entering the left ventricle, the oxygen demand of the heart muscle as a whole cannot be determined. However, from the absorption of oxygen from the blood drained by the coronary sinus, a sufficiently complete picture can be obtained of the oxygen balance of the heart.

In our opinion the method suggested may be used for the study of the oxygen demand of other organs as well as the heart.

SUMMARY

The author proposes a method of determining oxygen intake by the heart in anesthetized cats. Determination of oxygen intake by cardiac muscle is based upon continuous determination of oxyhemoglobin content in the blood flowing from the coronary sinus. Modified Krep's oximeter is employed for this purpose.

LITERATURE CITED

[1] N. V. Kaverina, Farmakol. i. Toksikol. 21, 39 (1958).

[2] D. L. Drabkin and C. F. Schmidt, J. Biol. Chem 157, 69 (1945).

[3] L. N. Katz, Ann New York Acad. Sci. 64, 4, 505 (1956).

[4] K. Kramer, Ztschr. f. Biol. 96, 61 (1935).

[5] D. Laurent, C. Bolene Williams, F. L. Williams and L. N. Katz, Am. J. Physiol. 185, 355 (1956).

[6] I. C. Roddie, J. T. Shepherd and R. F. Whelan, Brit. Heart J. 19, 516 (1957).

[7] E. Watkins, Proc. Soc. Exp. Biol. Med. 72, 180 (1949).

[8] E. H. Wood, J. E. Geraci and A. A. Pollack, Proc. Staff Meet Mayo Clin. 23, 494 (1948).

THE DETECTION OF DEAD AND LIVING CELLS BY THE METHOD OF FLUORESCENT MICROSCOPY

P. V. Breivis and Yu. M. Vasil'ev

From the Laboratory of Chemotherapy (Head - Corresponding Member AMN SSSR Prof. L. F. Larionov) and the Laboratory of Tumor Strains (Head - Dr. Biol. Sci. E. E. Pogosyants; Head of Division - Active Member AMN SSSR A. D. Timogeevskii) of the Institute of Experimental Pathology and Therapy of Cancer (Director - Corresponding Member AMN SSSR Prof. N. N. Blokhin) of the AMN SSSR, Moscow

(Received May 16, 1958. Presented by Active Member AMN SSSR A. D. Timofeevskii)

The problem of differentiation of dead and living cells is a very important one in various biological fields. For this reason the development of methods to do this deserves special attention. As long ago as 1940, Strugger [20] and also Bukatsch and Haitinger [11] suggested using for this purpose the fluorochrome stain acridine orange (AO), which they had produced. Strugger developed a test of viability in cells of plants, bacteria and yeasts. From his results he concluded that living cells fluoresce a green color and dead cells red, with transitional forms orange. Strugger's findings were confirmed in investigations by other workers [13-17, 19, 21] on various biological objects.

At the same time, the test proposed by Strugger was subjected to severe criticism in papers by many other workers who concluded that this method cannot be used to determine the viability of cells [2, 4-6, 9, 10, 12, 18]. G. A. Medvedeva [3], in M.N. Meisel's laboratory, proposed the use of the acid fluorochrome dye primulin in distinguishing living and dead cells of yeasts.

In order to ascertain the suitability of acridine orange and primulin for the detection of living and dead animal cells, we carried out experiments with ascitic cells from an Ehrlich's tumor exposed to various inactivating agents. The work was done in conformity with all the conditions of fluorochrome technique as given in the paper by Vinegar [21], who concluded that AO could be used for determining the viability of animal cells.

EXPERIMENTAL METHOD

All the experiments using inactivating agents and the control experiments were carried out with one sample of ascitic fluid.

This fluid was first diluted 1:100 with phosphate buffer (pH = 6), after which the concentration of cells in such a suspension was determined in a Goryaev counting chamber. This concentration was found to be 75×10^6 cells per cm^3. Phosphate buffer was then added to the suspension in a volume calculated to give a suspension containing 10^5 cells per cm^3. The suspension obtained was poured into tubes and used for the experiments with the inactivating agents. The following series of experiments were carried out.

1. Control series. 1 cm^3 of the suspension was inoculated (i.e. 10^5 cells) without additional treatment.

2. Inactivation by high temperature. Before inoculation, the tube with the suspension was placed on a water bath at a temperature of 60° for 3 minutes.

3. Irradiation with x-rays. Irradiation was with an RUM-3 apparatus at a temperature of 0° and with the following technical conditions: voltage 180 kv, current 10 ma, focal distance 30 cm, filters 0.5 Cu + 1.0 Al; total dose 3000 r.

Relative Number (in per cent) of Tumor Cells of Various Types of Fluorescence after Fluorochrome Staining and the Successful Innoculation of a Cell Suspension After Treatment with Various Agents

Experimental series	Successful inoculation of ascitic carcinoma*	Mean time of death of mice from ascitic carcinoma in days	Acridine orange			Primulin + AO		
			I	II	III	I	II	III
			solitary red granules or absence of granules	large number of red granules or diffusely pink cytoplasm	cytoplasm full of large granules of diffusely red	very faint dull green fluorescence of cytoplasm	yellowish pink cytoplasm	yellow-green fluorescence of the whole cell
1. Control (with 100,000 tumor cells)	20/20	8.9 ± 0.3	30.0	40.5	29.5	50.4	48.0	1.6
2. Inactivation by high temperature	0/10	–	18.0	63.0	19.0	8.0	10.0	82.0
3. Irradiation with x-rays	0/10	–	19.2	56.8	25.0	25.0	11.6	80.3
4. Inactivation with alcohol	0/10	–	16.0	60.2	23.8	1.0	6.6	82.4
5. Autolysis at 37° for 10 hours	4/10	38 ± 5.1	23.2	51.6	25.2	23.0	19.0	58.0
6. Autolysis at 37° for 24 hours	0/10	–	21.8	52.2	26.0	25.0	14.0	61.0
7. Inactivation by sarcolysin for 10 hours	9/10	18.4 ± 1.2	13.8	61.2	25.0	38.0	50.0	12.0
8. Inactivation with sarcolysin for 24 hours	7/10	27.7 ± 0.8	11.7	63.0	25.3	21.0	33.0	46.0
9. Control of the experiments with sarcolysin and autolysis	10/10	11.6 ± 0.5	28.0	42.8	29.2	54.0	44.0	2.0

* The denominator is the number of mice in the experiment, the numerator the number of mice in which an ascitic carcinoma developed.

4. Inactivation with alcohol. The suspension was mixed with 30° alcohol in a proportion of 1:1 and incubated for 10 minutes at +4°, then centrifuged for 2 minutes at 1000 rpm, after which the precipitated cells were resuspended in phosphate buffer.

5-6. Autolysis. The suspension was incubated at 37° for 10 or 24 hours.

7-8. Inactivation by sarcolysin. A solution of sarcolysin in phosphate buffer (100γ/ml) was mixed with the suspension of ascitic cells in a proportion of 1:1. The mixture was incubated for 10 or 24 hours, after which it was centrifuged and the precipitated cells were resuspended in phosphate buffer.

9. Control series to the experiments with autolysis and sarcolysin. The suspension was incubated for 24 hours at 4°.

After the inactivating agents had been applied, 2 ml of suspension was taken from each tube for examination under the fluorescent microscope. Fluorochrome staining with AO was carried out, 1 ml of a 2×10^{-4} solution of AO being added to 1 ml of the cell suspension. A study of the fluorescent microscopic picture of the ascitic cells, stained with AO, enabled them to be divided into 3 groups according to the nature of fluorescence of their cytoplasm: 1) the cytoplasm was a dull green color with solitary red granules; 2) the cytoplasm was diffusely pink in color, or had a large number of red granules; 3) the cytoplasm was diffusely red or filled with very red granules.

On fluorochrome staining with primulin, the living ascitic cells did not show up. For this reason, to reveal the outlines of the cells (nucleus and cytoplasm) we added to the primulin solution (1:10,000) a few drops of AO in the same concentration. To 1 ml of cell suspension 1 ml of 2×10^{-4} primulin was added. By this method of fluorochrome staining the ascitic cells were divided into 3 groups according to the nature of their fluorescence: I, dull green cytoplasm, green nucleus; II, yellowish pink cytoplasm, green nucleus; III, yellowish green fluorescence of the whole cell (nucleus and cytoplasm difficult to distinguish).

During all the cell counts the relative number of cells of each group in 1000 tumor cells was determined. The material from each experimental series was inoculated intraperitoneally into 10 mice; all the experimental mice received the same volume of suspension (1 ml) as the control mice (see above). The mice were observed for 2 months after inoculation.

EXPERIMENTAL RESULTS

The results of the experiments are shown in the Table.

It must be pointed out that we judged the degree of inactivation of the ascitic tumor cells both by the decrease in the percentage of successful inoculations of the tumor and by the changes in the time of death of the mice from ascitic carcinoma. We know from the literature [1, 21 and others] that both of these indices are functions of the number of viable ascitic carcinoma cells injected. As may be seen from the table, in the control series (series 1 and 9) the ascitic carcinoma took successfully in all the animals and death ensued after a short time (in the second week of the experiment). The inactivating agents used could conventionally be divided into 2 categories by the degree of inhibition of growth of the Ehrlich's carcinoma. The "weak" inactivating agents included those which lowered the percentage of successful inoculations of the tumor and retarded its growth. These were autolysis at 37° for 10 hours (series 5) and incubation of the tumor cells mixed with sarcolysin for 10 or 24 hours (series 7 and 8). The "strong" inactivating agents were those which caused the percentage of successful inoculations of a suspension of ascitic cells to fall to zero. These were high temperature (series 2), irradiation by x-rays (series 3), the action of alcohol (series 4) and autolysis at 37° for 24 hours (series 6).

Let us compare the results of the experiments in which suspensions of ascitic cells were inoculated after the application of various agents and the results of the counts of the relative numbers of cells with the various types of fluorescence after fluorochrome staining of the same suspensions with AO. The only more or less regular difference between the control and experimental series was a slight increase in the number of group II cells (with a large number of granules or diffusely pink cytoplasm) and a fall in the number of group I cells after the use of the inactivating agents. This increase, however, was not very significant (roughly from 40 to 50-60%) and was not proportional to the degree of inactivation of the ascitic cells. The relative number of group III cells in general was hardly changed.

We did not therefore observe that marked alteration in the fluorescence of the cells which might have been expected from Strugger's ideas, i.e. the appearance of a red fluorescence of the cytoplasm instead of green in response to the inactivating agents. Both normally and after inactivation tumor cells with differing amounts of fluorescent cytoplasmic substance could be seen in the suspension. Our experiments agreed well with the conclusions of M. N. Meisel' and B. V. Korchagina [7] and of certain other workers [8, 9], who showed that this red fluorescence was characteristic of structures containing ribonucleic acid. Some experiments which we performed in fact showed that by the action of crystalline ribonuclease (1 mg enzyme to 1 ml of distilled water; incubation for 1 hour at 37°) on films of ascitic carcinoma, previously fixed with Carnoy's fluid and stained with fluorochrome AO, a substance with a red fluorescence completely disappeared from the cytoplasm of the tumor cells. Hence it could be concluded that the changes which we mentioned above in the relative content of cells with various types of fluorescence evidently reflect changes caused by the inactivating agents in the ribonucleic acid content of the cytoplasm of the ascitic cells. As may be seen from the above, examination of the fluorescence of cells stained with AO cannot be used as a method of detecting living and dead cells in a suspension, although it is a very valuable method of study of the nucleic acids of the cell during life.

Different results are given by an analysis of the data of examination of the fluorescence of the same cell suspensions after fluorochrome staining with primulin. Particularly demonstrative changes were found here in the relative number of group III cells, intensively combining with the fluorochrome, and hence giving an intense yellow-green fluorescence of the whole body of the cell. In the control series these cells did not account for more than 1-2% of all the cells; after the use of "weak" inactivating agents the number of group III cells rose to 12-58%. Under these circumstances in the series of experiments in which the degree of inactivation of the tumor cells was least (after incubation with sarcolysin for 10 hours; series 7) the group III cells were only 12% of the total number. At the same time, when the length of incubation was increased to 24 hours (series 8), and the inactivation became more marked, the percentage of these cells rose to 46. After autolysis for 10 hours (series 5), causing still more marked inactivation of the cells, the number of group III cells reached 58%. Finally, in all the experiments using "strong" inactivating agents (series 2, 3, 4, 6) the group III cells accounted for 61-82% of the cells in the suspension.

Thus the percentage of cells intensively accumulating primulin changes in parallel with the degree of inactivation of the ascitic carcinoma cells. There are, therefore, grounds for considering that the cells of this category are inactivated. It is difficult to determine the character of the group II cells with their weak yellowish pink fluorescence of the cytoplasm. There are many such cells in the ascitic cell suspensions of both experimental and control series; these cells may possibly be completely viable, but we cannot yet be sure that among them a variable number of injured cells may not be present. It follows from the above that examination by the fluorescent microscope of cells stained with primulin can be used as a rough guide to the determination of the relative number of living and dead cells in cell suspensions. It is not yet clear at what stage of injury to the cell the accumulation of fluorochrome begins. The problem of whether only cells which have irreversibly lost their viability may be detected in this way therefore requires further study.

SUMMARY

Results of fluorescent microscopic investigation of suspension of Ehrlich ascitic carcinoma cells treated in vitro with different inactivating agents were compared with results of biological experiments by which the viability of these cells has been tested. It was concluded that the examination of the fluorescence of cells stained with acridine orange cannot be used for differentiation of the living from the dead cells. At the same time this technique may serve as a valuable method for supravital examination of both nucleic acids. Examination of the fluorescence of cells stained with primuline can be used for the approximate estimation of the percentage of viable cells in the cell suspensions.

LITERATURE CITED

[1] Yu. M. Vasil'ev, Byull. Éksptl. Biol. i Med. 5, 379-382 (1950).

[2] N. A. Krasil'nikov and M. N. Bekhtereva, Mikrobiologiya, 25, 3, 279-285 (1956).

[3] G. A. Medvedeva, Vestnik Akad. Nauk SSSR, 9, 88-89 (1951).

[4] M. N. Meisel', Mikrobiologiya 16, 6, 527-535 (1947).

[5] M. N. Meisel', Vestnik Akad. Nauk SSSR, 9, 87 (1951).

[6] M. N. Meisel' and N. B. Zavarzina, Mikrobiologiya 16, 5, 394-402 (1947).

[7] M. N. Meisel' and B. V. Korchagin, Byull. Eksptl. Biol. i Med. 3, 49-52 (1952).

[8] J. Armstrong, Exper. Cell Res. 11, 640-643 (1956).

[9] L. Bertallanffy and I. Bickis, J. Histochem. 4, 481 (1956).

[10] H. Bogen, Arch. Microbiologie. 18, 170-173 (1941).

[11] F. Bukatsch and M. Haitinger, Protoplasma 34, 515-519 (1940).

[12] F. Dangl, Mikrochemie 37, 1163-1168 (1951).

[13] C. Duijn, Mikroskopie 9, 324-333 (1954).

[14] K. Gartner, Zbl. Bakter. Abt. Orig. 150, 97-114 (1943).

[15] H. Koelbel, Ztschr. Naturforsch. 28, 382-387 (1947).

[16] A. Krebs, Strahlentherapie 75, 346-352 (1944).

[17] A. Krebs and S. Gierlach, Am. J. Roentgenol. 65, 93-96 (1951).

[18] J. May, Zbl. Bact. I Abt. Orig. 152, 586-599 (1948).

[19] W. Schummelfeder, Path. Anat. 318, 119-154 (1950).

[20] S. Strugger, Jenaische Ztschr. f. Naturwiss. 73, 2, 97-134 (1940).

[21] R. Vinegar, Cancer Res. 16, 9, 900-906 (1956).

A METHOD OF RECORDING THE BALLISTOCARDIOGRAM OF ACCELERATION

I. E. Oranskii and N. F. Smirnov

From the Sverdlovsk Research Institute of Spa Treatment and Physiotherapy
(Director - Candidate Med. Sci. N. V. Orlov, Scientific Director -
Dr. Med. Sci. D. G. Shefer)

(Received March 3, 1958. Presented by Active Member AMN SSSR V. V. Parin)

Direct ballistocardiography is nowadays widely used in the practice of hospitals and research institutes. The majority of electromagnetic recorders used for this purpose enable the ballistocardiogram of velocity to be traced. Recorders for tracing the ballistocardiogram of acceleration ("the one which most accurately reflects the propulsive function of the heart" – Dock) are complicated and so are less widely used in practice.

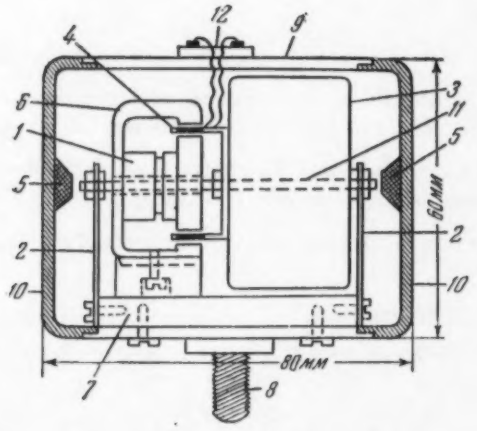


Fig. 1. General features of the recorder. 1) Magnetic ring; 2) flat steel springs; 3) seismic mass (brass); 4) coil; 5) limiting stops (rubber); 6) magnetic conductor (iron); 7) base (iron); 8) fixing bolt of the recorder (iron); 9-10) body and top of the recorder (iron); 11) bolt for the moving part (brass); 12) coil leads.

In the Sverdlovsk Research Institute of Spa Treatment and Physiotherapy an electrodynamic recorder has been constructed which enables the ballistocardiogram of acceleration to be recorded by a direct method.

In its construction the recorder (Fig. 1) consists of an electrodynamic system of a permanent ring magnet 1, taken from a type 0.5 GD dynamic loudspeaker, in the gap of which a coil 4 with a weight 3, acting as a seismic mass, are suspended on flat steel springs.

The coil has a large number of turns of PÉL 0.05 mm wire with a resistance of the order of 1 kohm. The coil leads are connected in parallel with a condenser with a capacity of 2 and 20 μf, which creates a constant period of 180-200 millisec.

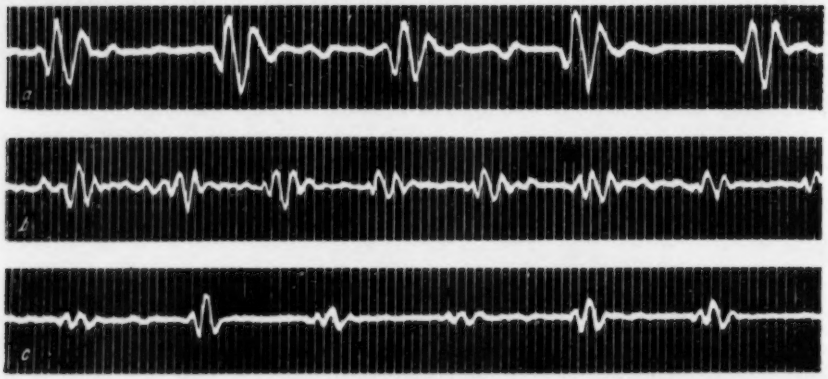


Fig. 2. Ballistocardiograms. a)Of a healthy person; zero degree of abnormality; b) patient N-va, aged 30 years; diagnosis hypertension, angina pectoris, first degree of abnormality; high L waves; c) patient U-v, aged 29 years; diagnosis hypertension, atherosclerosis second degree of abnormality.

The body 9 of the recorder is fixed to a strip with the screw 8 and placed on the subject's knees. In response to movement of the person's body, the body of the recorder together with the magnet move synchronously, but the coil, by virtue of the inertia of its mass, remains relatively at rest. In consequence of the displacement of the magnet in relation to the coil, an electrodynamic current is induced in the latter, the average value of which reaches 1-1.5 mv.

The tracing is made on an EKP 4 electrocardiograph with a rate of film movement of 4 cm/sec.

By means of this recorder ballistocardiograms were registered in persons of different ages and sex, both healthy and suffering from hypertension, atheromatous cardiosclerosis, angina pectoris, and organic heart disease.

The ballistocardiograms were interpreted by the method suggested by Brown, and in addition the curves were analyzed quantitatively (Dock, A. L. Myasnikov, V. V. Parin).

The results obtained agree completely with the clinical picture of the disease and the reports in the literature on this subject.

The recorder is portable and convenient to use.

By way of illustration we show curves traced by means of our recorder (Fig. 2).

SUMMARY

The authors describe a new apparatus for recording the ballistocardiogram of acceleration which has been designed at the Sverdlovsk Scientific Research Institute of Spa Treatment and Physiotherapy.

The new design of electrodynamic recorder permits registration of ballistocardiogram of acceleration by direct method.

In comparison with the known models the new apparatus is portable and convenient. The results of investigation obtained with its aid completely correspond to the clinical picture of the disease.

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiia	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiia	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Embriol.	Arkhiv Anatomii Gistologii i Embriologii	Archives of Anatomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiia	Biokhimiia	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiia Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Biull. Eksptl. Biol. i Med.	Biulleten Eksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Biull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Biulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Eksptl. Khirurg.	Eksperimentalnaia Khirurgiia	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiia i Toksikologiia	Pharmacology and Toxicology
Farmatsiia	Farmatsiia	Pharmacy
Fiziol. Rastenii	Fiziologiia Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenova Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariia	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiia Akademii Nauk SSSR, Seriia Biologicheskaia	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiia Tikhookeanskogo N. I. Instituta Rybnogo Khoziaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiia	Khirurgiia	Surgery
Klin. Med.	Klinicheskaia Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo(po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaia Parazitologiia i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaia Radiologiia	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiia	Mikrobiologiia	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhiat. i Psikhogig.	Nevropatologiia, Psikhiatriia i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediia, Travmatologiia i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriia	Pediatriia	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Endokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniia Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaia Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journals
Stomatologiia	Stomatologiia	Stomatology
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriia	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics

* LIBRARY OF CONGRESS TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiia	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiia	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiia, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoiuz. Gidrobiol. Obshchestva	Trudy Vsesoiuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoiuz. Inst. Ekspti. Med.	Trudy Vsesoiuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiia	Urologiia	Urology
Uspekhi Biokhimiia	Uspekhi Biokhimiia	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiia	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriia Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universiteta, Seriia Biologii i Pochvovedeniia	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiia	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariia	Veterinariia	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirurgii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniia	Voprosy Pitaniia	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaia Laboratoriia	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiia	Journal of General Biology
Zhur. Vysshei Nerv. Deiatel.	Zhurnal Vysshei Nervnoi Deiatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

For the first time in English . . . .

RESEARCH ON NOVOCAIN THERAPY IN OLD AGE

American researchers and physicians can now judge for themselves the significance of the dramatic results of novocain therapy in maladies of old age, as reported by Dr. Anna Aslan, *Director of the Institute of Geriatrics, Bucharest, Rumania,* and her co-workers.

These seven papers, now in complete English translation, originally appeared in the West German journal *"Die Therapiewoche."* The authors report the results of large-scale clinical application of novocain injections for treatment of the sequelae of old age. The research has continued over a seven-year period and has covered more than 5,000 people. Dr. Aslan and her colleagues claim to have achieved a reversal of phenomena previously considered irreversible, e.g., in *cerebral arteriosclerosis;* they report sustained improvement in senile Parkinsonism, ichthyosis and senile Keratosis, and a reduction in arteriosclerotic symptons.

These reports have been translated and published in English so that they can be more readily subjected to objective analysis and evaluation by qualified scientists and physicians.

TITLES OF REPORTS

A New Method for Prophylaxis and Treatment of Aging with Novocain—Eutrophic and Rejuvenating Effects • Prevention and Treatment of Diseases of the Aged—Views Concerning Treatment of Old Age • Biochemical and Physiological Criteria of Aging and Effects Thereon by Treatment, as a Measure of Rehabilitation • Recent Experiences on the Rejuvenating Effect of Novocain Together with Experimental, Clinical and Statistical Proof • Results of Treatment with Novocain in Dysmetabolic Arthropathies • Significance of Biomorphosis for Aging, Disease and Rejuvenation • First Experiences with Novocain Therapy of Old People.

The Complete Collection $12.50

CONSULTANTS BUREAU, INC.

227 WEST 17TH STREET, NEW YORK 11, N. Y

THOUSANDS OF JOURNALS

throughout the world are publishing significant research papers in

Experimental Biology and Medicine

any one of which might be of vital interest to you

How do you keep posted on current advances in your field? No individual possibly could read it all in the original even if the journals were available to him. *Biological Abstracts* does this arduous task for you. Currently publishing more than 40,000 abstracts of research papers in biology, it plans gradually to expand its coverage to 80,000 within the next ten years in order to keep pace with the ever-increasing output of biological literature.

Biological Abstracts, a cooperative, non-profit undertaking, is published by biologists for biologists in a complete edition covering all fields of biology, and five low-priced sectional editions that are specially designed for individual use. Two of these sectional editions have particular value for those working in Experimental Biology and Medicine:

SECTION B—each month 1,200 to 1,500 abstracts of research papers in Animal Physiology, Pharmacology, Morphology and Pathology, exclusive of Microbiology, are published.

SECTION C—each month some 450 to 550 research papers in Microbiology, including General Bacteriology, Medical Bacteriology, Viruses, Pathogenic Fungi, Chemotherapy, Immunology, Public Health, Industrial Microbiology, and Parasitology are abstracted.

Individuals are allowed a discount of 50% off the list prices of the sectional editions if the subscriptions are for their personal use.

Sample copies and complete information will be sent upon request.

BIOLOGICAL ABSTRACTS, University of Pennsylvania
3815 Walnut Street, Philadelphia 4, Pa.